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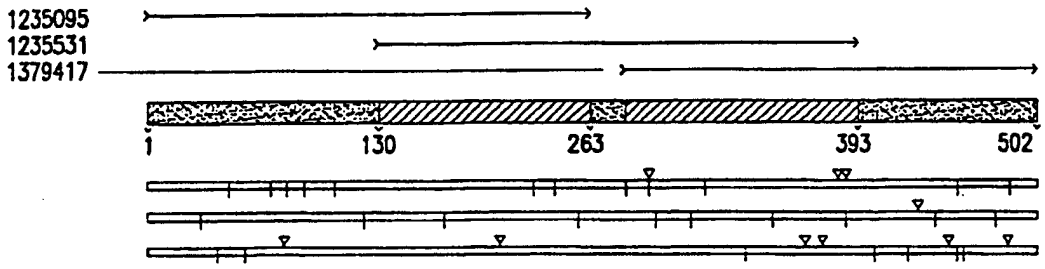

(57) Abstract

A set of contiguous and partially overlapping RNA sequences and polypeptides encoded thereby, designated as LU103 and transcribed from lung tissue is described. A fully sequenced clone representing the longest continuous sequence of LU103 is also disclosed. These sequences are useful for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition of an individual to diseases and conditions of the lung such as lung cancer.

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REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE LUNG

Background of the Invention

The invention relates generally to detecting diseases of the lung. More particularly, the invention relates to reagents such as polynucleotide sequences and the polypeptide sequences encoded thereby, as well as methods which utilize these sequences. The polynucleotide and polypeptide sequences are useful for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining predisposition to diseases or conditions of the lung such as lung cancer.

Lung cancer is the second most common cancer for both men and women in the United States, with an estimated 178,100 newly diagnosed during 1997 (American Cancer Society statistics). It also is the most common cause of cancer death for both sexes, with over 160,000 lung cancer related deaths expected in 1997. Lung cancer is a major health problem in other areas of the world, with approximately 135,000 new cases occurring each year in the European Union, and its incidence rapidly increasing in Central and Eastern Europe. See, Genesis Report, February 1995 and T. Reynolds, J. Natl. Cancer Inst. 87: 1348-1349 (1995).

Early stage lung cancer can be detected by chest radiograph and the sputum cytological examination; however, these procedures do not have sufficient sensitivity for routine use as screening tests for asymptomatic individuals. Potential technical problems which can limit the sensitivity of chest radiograph include suboptimal technique, insufficient exposure, and positioning and cooperation of the patient. T.G. Tape et al., Ann. Intern. Med. 104: 663-670 (1986). Moreover, radiologists often disagree on interpretations of chest radiographs; over 40% of these disagreements are significant or potentially significant, with false-negative interpretations being the cause of most errors. P.G. Herman et al., Chest 68: 278-282 (1975). Inconclusive results require additional follow-up testing for clarification. T.G. Tape et al., supra. Sputum cytology is even less sensitive than

chest radiography in detecting early lung cancer; of 160 lung cancer cases, radiography alone detected 123 cases (77%) while cytological examination alone detected 67 cases (42%). The National Cancer Institute "Early Lung Cancer Detection: Summary and Conclusion," Am. Rev. Resp. Dis. 130: 565-567 (1984). Factors affecting the ability of sputum cytological examination to diagnose lung cancer include the ability of the patient to produce sufficient sputum, the size of the tumor, the proximity of the tumor to major airways, the histologic type of the tumor, and the experience and training of the cytopathologist. R.J. Ginsberg et al. In: Cancer: Principles and Practice of Oncology, Fourth Edition, V.T. DeVita, S. Hellman, S.A. Rosenburg, pp. 673-723, Philadelphia, PA: J.B. Lippincott Co. (1993).

A majority of new lung cancers are being detected only when the disease has spread beyond the lung. In the United States only 16% of new non-small cell lung cancers are detected at a localized stage when 5-year survival is highest (at 49.7%). In contrast, 68% of new cases are detected when the disease has already spread locally (regional disease) or metastasized to distant sites (distant disease) which have significantly lower 5-year survival rates of only 18.5% and 1.8%, respectively. Similarly, 80% of newly detected small-cell lung cancers are discovered with regional disease or distant disease, which have 5-year survival rates of only 9.5% and 1.7%, respectively. Stat Bite, J. Natl. Cancer Inst. 87: 1662, 1995. Thus current procedures fail to detect lung cancer at an early, treatable stage of the disease. Improved methods of detection therefore are needed to reduce mortality.

After diagnosis, the patient's cancer is staged. Staging is a strong predictor of patient outcome and determines the treatment regimen for the patient. Patients with cell lung cancer can undergo routine CT scanning of the chest and upper abdomen in an effort to detect lymph node metastasis, pulmonary metastases, and liver and adrenal metastases. The results of this CT scan frequently are inconclusive and lead to additional testing, including bone scans. Staging of patients may also include bone scans, fiberoptic bronchoscopy with bronchial washings, in addition to biopsy and liver function tests.

The most frequently used methods for monitoring lung cancer patients after primary therapy are clinic visits, chest X-rays, complete blood counts, liver function tests and chest CT scans. Detecting recurrence by such monitoring techniques, however, does not greatly affect mode of treatment and overall survival time. This leads to the conclusion that current monitoring methods are not cost effective. K.S.

Naunheim et al., Ann. Thorac. Surg. 60: 1612-1616 (1995). G. L. Walsh et al., Ann. Thorac. Surg. 60: 1563-1572 (1995).

Attempts have been made to discover improved tumor markers for lung cancer by first identifying differentially expressed cellular components in lung tumor tissue compared to normal lung tissue. For example, two-dimensional polyacrylamide gel electrophoresis has been used to characterize quantitative and qualitative differences in polypeptide composition. T. Hirano et al., Br. J. Cancer 72: 840-848 (1995); A.T. Endler et al., J. Clin. Chem Clin. Biochem. 24:981-992 (1986). The sensitivity of this technique is limited, however, by the degree of protein resolution of the two electrophoretic steps and by the detection step. This step depends on staining protein in gels. The polypeptide instability may generate artifacts in the two-dimensional pattern. Another technique, subtractive hybridization, has been used to screen for differences in gene expression between normal and tumor tissue. P.S. Steeg et al., J. Natl. Cancer Inst. 80: 200-204 (1988). This technique is laborious and has limitations in detecting mRNA species in tissues present in low amounts. A more sensitive method for identifying differentially expressed genes is differential display. P. Liang et al., Cancer Res. 52:6966-6968 (1992). This method involves the reverse transcription of cellular mRNAs to cDNAs followed by PCR amplification of a cDNA subpopulation. Comparison of amplified cDNA subpopulations between normal and tumor lung tissues allows identification of mRNA species that are differentially expressed. This technique has greater sensitivity than subtractive hybridization for detecting mRNAs of low abundance, but is a difficult technique to perform in a routine clinical laboratory and therefore is confined to the research setting. A novel gene termed N8 recently was found by differential display to express higher levels of mRNA in lung tumor than in normal lung tissue. S.L. Chen et al., Oncogene 12: 741-751 (1996). However, no marker currently is available for use in routine screening assay techniques, such as immunological assays. Tests based upon the appearance of various markers in test samples such as blood, plasma or serum and detectable by such immunological methods could provide low-cost, non-surgical, diagnostic information to aid the physician to make a diagnosis of cancer, help stage a patient, select a therapy protocol or monitor the success of the chosen therapy.

Such markers have been placed into several categories. The first category contains those markers which are elevated in disease. Examples include chorionic gonadotropin (HCG) which is elevated in testicular cancer and alpha fetoprotein

(AFP) which is elevated in hepato-cellular carcinoma (HCC). E.L. Jacobs, Curr. Probl. Cancer 15 (6): 299-350 (1991). The second category contains those markers which are altered in disease. Examples include splice variants of CD44 in bladder cancer Y. Matsumura et al., Journal Pathology 175 (Suppl): 108A (1995) and mutations in p53 in lung and colorectal cancer. W.P. Bennett, Cancer Detection and Prevention 19 (6): 503-511 (1995). In the latter case, p53 mutations result in a protein which is defective in function and which may or may not be detectable by assays based on function or specific antibodies directed against the native protein. The third category contains those markers which are normal proteins but which appear in an inappropriate body compartment. Examples include prostate specific antigen (PSA) which is a normal protein secreted at high levels into the seminal fluid, but which is present in very low levels in the blood of men with normal prostates. P.H. Lange et al., Urology 33 (6 Suppl): 13 (1989). However, in patients with diseases of the prostate, including benign prostatic hyperplasia (BPH) or adenocarcinoma of the prostate, the level of PSA is markedly elevated in the blood and is a strong indication of disease of the prostate. Similarly, carcinoembryonic antigen (CEA) is a normal component of the inner lining of the colon and is present in blood only at low levels in people without diseases of the colon. E.L. Jacobs, supra. However, in diseases of the colon including inflammatory bowel disease and adenocarcinoma of the colon, the concentration of CEA is markedly elevated in the blood plasma or serum of many patients and is an indicator of disease in the tissue. It also has been recognized that while CEA and PSA are produced in some tissues other than the colon or prostate, respectively, these markers still are useful in the diagnosis of disease of their primary tissue of origin due to their strong tissue selectivity.

There are yet other examples of inappropriate compartmentalization of markers. For example, in the case of metastatic cancer, lymph nodes often contain cells which have originated from the primary tumor and which often express immunohistochemical markers of the primary tumor. CEA and PSA both have been detected in the lymph nodes of patients with metastisized cancer. Other compartments in which the inappropriate appearance of normal gene products are indicative of disease include the formed elements of whole blood, which are thought to provide evidence of the metastatic spread of the disease. To date, however, no such marker for the screening or diagnosis of lung diseases such as lung cancer, asthma and adult respiratory distress syndrome exists.

It therefore would be advantageous to provide methods and reagents for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to diseases and conditions of the lung such as lung cancer. Such methods would include assaying a test sample for products of a gene (or genes) which are overexpressed in diseases and conditions associated with lung cancer. Such methods may also include assaying a test sample for products of a gene (or genes) which have been altered by the diseases and conditions associated with lung cancer. Such methods may further include assaying a test sample for products of a gene (or genes) whose distribution among the various tissues and compartments of the body have been altered by the diseases and conditions associated with lung cancer. Such methods would comprise making cDNA from mRNA in the test sample, amplifying (when necessary) portions of the cDNA corresponding to the gene or a fragment thereof, and detecting the cDNA product as an indication of the presence of the cancer; or detecting translation products of the mRNAs comprising the gene sequence(s)as an indication of the presence of the disease. These reagents include polynucleotide(s) or fragment(s) thereof which may be used in diagnostic methods such as reverse transcriptase-polymerase chain reaction (RT-PCR), polymerase chain reaction (PCR), or hybridization assays of biopsied tissue; polypeptides which are the translation products of such mRNAs; or antibodies directed against these proteins. Such methods would include assaying a sample for product(s) of the gene and detecting the product(s) as an indication of lung cancer. Drug treatment or gene therapy for lung diseases such as lung cancer can be based on these identified gene sequences or their expressed polypeptides, and efficacy of any particular therapy can be monitored using the diagnostic methods disclosed herein. Furthermore, it would be advantageous to have available alternate diagnostic methods capable of detecting early lung cancer in a non-invasive manner. Also of benefit would be methods to stage and monitor the treatment of lung disease.

Summary of the Invention

The present invention provides a method of detecting a target LU103 polynucleotide in a test sample which comprises contacting the test sample with at least one LU103-specific polynucleotide and detecting the presence of the target LU103 polynucleotide in the test sample. The LU103-specific polynucleotide has at

least 50% identity with a polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof. Also, the LU103-specific polynucleotide may be attached to a solid phase prior to performing the method.

The present invention also provides a method for detecting LU103 mRNA in a test sample, which comprises performing reverse transcription (RT) with at least one primer in order to produce cDNA, amplifying the cDNA so obtained using LU103 oligonucleotides as sense and antisense primers to obtain LU103 amplicon, and detecting the presence of the LU103 amplicon as an indication of the presence of LU103 mRNA in the test sample, wherein the LU103 oligonucleotides have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof. Amplification can be performed by the polymerase chain reaction. Also, the test sample can be reacted with a solid phase prior to performing the method, prior to amplification or prior to detection. This reaction can be a direct or an indirect reaction. Further, the detection step can comprise utilizing a detectable label capable of generating a measurable signal. The detectable label can be attached to a solid phase.

The present invention further provides a method of detecting a target LU103 polynucleotide in a test sample suspected of containing target LU103 polynucleotides, which comprises (a) contacting the test sample with at least one LU103 oligonucleotide as a sense primer and at least one LU103 oligonucleotide as an anti-sense primer, and amplifying same to obtain a first stage reaction product; (b) contacting the first stage reaction product with at least one other LU103 oligonucleotide to obtain a second stage reaction product, with the proviso that the other LU103 oligonucleotide is located 3' to the LU103 oligonucleotides utilized in step (a) and is complementary to the first stage reaction product; and (c) detecting the second stage reaction product as an indication of the presence of a target LU103 polynucleotide in the test sample. The LU103 oligonucleotides selected as reagents in the method have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof. Amplification may be performed by the polymerase chain reaction. The test sample can be reacted either directly or indirectly with a solid phase prior to

performing the method, or prior to amplification, or prior to detection. The detection step also comprises utilizing a detectable label capable of generating a measurable signal; further, the detectable label can be attached to a solid phase. Test kits useful for detecting target LU103 polynucleotides in a test sample are also provided which comprise a container containing at least one LU103-specific polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof. These test kits further comprise containers with tools useful for collecting test samples (such as, for example, blood, urine, saliva and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing urine or stool samples. Collection materials, such as papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens.

The present invention provides a purified polynucleotide or fragment thereof derived from an LU103 gene. The purified polynucleotide is capable of selectively hybridizing to the nucleic acid of the LU103 gene, or a complement thereof. The polynucleotide has at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof. Further, the purified polynucleotide can be produced by recombinant and/or synthetic techniques. The purified recombinant polynucleotide can be contained within a recombinant vector. The invention further comprises a host cell transfected with said vector.

The present invention further provides a recombinant expression system comprising a nucleic acid sequence that includes an open reading frame derived from LU103. The nucleic acid sequence has at least 50% identity with a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof. The nucleic acid sequence is operably linked to a control sequence compatible with a desired host. Also provided is a cell transfected with this recombinant expression system.

The present invention also provides a cell transfected with an LU103 nucleic acid sequence that encodes at least one epitope of an LU103 antigen, or fragment thereof. The nucleic acid sequence is selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

Also provided is a composition of matter that comprises an LU103 polynucleotide of at least about 10-12 nucleotides having at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof. The LU103 polynucleotide encodes an amino acid sequence having at least one LU103 epitope. Also provided is a gene, or fragment thereof, coding for an LU103 polypeptide which has at least 50% identity to SEQUENCE ID NO 14; and a gene, or a fragment thereof, comprising DNA having at least 50% identity to SEQUENCE ID NO 4 or SEQUENCE ID NO 5.

Brief Description of the Drawings

FIGURE 1 shows the nucleotide alignment of clones 1235095 (SEQUENCE ID NO 1), 1235531 (SEQUENCE ID NO 2), 1379417 (SEQUENCE ID NO 3), and the consensus sequence (SEQUENCE ID NO 4) derived therefrom;

FIGURE 2 shows the contig map depicting the formation of the consensus nucleotide sequence (SEQUENCE ID NO 4) from the nucleotide alignment of overlapping clones 1235095 (SEQUENCE ID NO 1), 1235531 (SEQUENCE ID NO 2), and 1379417 (SEQUENCE ID NO 3);

FIGURE 3A is a scan of an ethidium bromide stained agarose gel of RNA from various tissue extracts and the corresponding northern blot of RNA using LU103 radiolabeled probe; FIGURE 3B is a scan of an ethidium bromide stained agarose gel of RNA from various lung tissues and the corresponding northern blot of RNA using LU103 radiolabeled probe;

FIGURE 4A is a scan of an ethidium bromide stained agarose gel of LU103-specific and RNA control-specific primed PCR amplification products from lung tissue RNAs; FIGURE 4B is a scan of an ethidium bromide stained agarose gel of LU103-specific primed PCR amplification products from RNAs of lung, prostate, breast and colon tissues.

Detailed Description of the Invention

The present invention provides a gene or a fragment thereof which codes for an LU103 polypeptide having at least about 50% identity to SEQUENCE ID NO 14. The present invention further encompasses an LU103 gene or a fragment thereof comprising DNA which has at least about 50% identity to SEQUENCE ID NO 4 or SEQUENCE ID NO 5.

The present invention provides methods for assaying a test sample for products of a lung tissue gene designated as LU103, which comprises making cDNA from mRNA in the test sample, and detecting the cDNA as an indication of the presence of lung tissue gene LU103. The method may include an amplification step, wherein one or more portions of the mRNA from LU103 corresponding to the gene or fragments thereof, is amplified. Methods also are provided for assaying for the translation products of LU103. Test samples which may be assayed by the methods provided herein include tissues, cells, body fluids and secretions. The present invention also provides reagents such as oligonucleotide primers and polypeptides which are useful in performing these methods.

Portions of the nucleic acid sequences disclosed herein are useful as primers for the reverse transcription of RNA or for the amplification of cDNA; or as probes to determine the presence of certain mRNA sequences in test samples. Also disclosed are nucleic acid sequences which permit the production of encoded polypeptide sequences which are useful as standards or reagents in diagnostic immunoassays, as targets for pharmaceutical screening assays and/or as components or as target sites for various therapies. Monoclonal and polyclonal antibodies directed against at least one epitope contained within these polypeptide sequences are useful as delivery agents for therapeutic agents as well as for diagnostic tests and for screening for diseases or conditions associated with LU103, especially lung cancer. Isolation of sequences of other portions of the gene of interest can be accomplished utilizing probes or PCR primers derived from these nucleic acid sequences. This allows additional probes of the mRNA or cDNA of interest to be established, as well as corresponding encoded polypeptide sequences. These additional molecules are useful in detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to, diseases and conditions of the lung such as lung cancer, characterized by LU103, as disclosed herein.

Techniques for determining amino acid sequence "similarity" are well-known in the art. In general, "similarity" means the exact amino acid to amino acid

comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more polynucleotide sequences can be compared by determining their "percent identity." Two or more amino acid sequences likewise can be compared by determining their "percent identity." The programs available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI), for example, the GAP program, are capable of calculating both the identity between two polynucleotides and the identity and similarity between two polypeptide sequences, respectively. Other programs for calculating identity or similarity between sequences are known in the art.

The compositions and methods described herein will enable the identification of certain markers as indicative of a lung tissue disease or condition; the information obtained therefrom will aid in the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining diseases or conditions associated with LU103, especially lung cancer. Test methods include, for example, probe assays which utilize the sequence(s) provided herein and which also may utilize nucleic acid amplification methods such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), and hybridization. In addition, the nucleotide sequences provided herein contain open reading frames from which an immunogenic epitope may be found. This epitope is believed to be unique to the disease state or condition associated with LU103. It also is thought that the polynucleotides or polypeptides and protein encoded by the LU103 gene are useful as a marker. This marker is either elevated in disease such as lung cancer, altered in disease such as lung cancer, or present as a normal protein but appearing in an inappropriate body compartment. The uniqueness of the epitope may be determined by (i) its immunological reactivity and specificity with antibodies directed against proteins and polypeptides encoded by the LU103 gene, and (ii) its nonreactivity with

any other tissue markers. Methods for determining immunological reactivity are well-known and include but are not limited to, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), hemagglutination (HA), fluorescence polarization immunoassay (FPIA), chemiluminescent immunoassay (CLIA) and others. Several examples of suitable methods are described herein.

Unless otherwise stated, the following terms shall have the following meanings:

A polynucleotide "derived from" or "specific for" a designated sequence refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the designated nucleotide sequence. The sequence may be complementary or identical to a sequence which is unique to a particular polynucleotide sequence as determined by techniques known in the art. Comparisons to sequences in databanks, for example, can be used as a method to determine the uniqueness of a designated sequence. Regions from which sequences may be derived, include but are not limited to, regions encoding specific epitopes, as well as non-translated and/or non-transcribed regions.

The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest under study, but may be generated in any manner, including but not limited to chemical synthesis, replication, reverse transcription or transcription, which is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived. As such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with the intended use.

A "fragment" of a specified polynucleotide refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the specified nucleotide sequence.

The term "primer" denotes a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target

nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase, RNA polymerase or reverse transcriptase.

The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, e.g., PNA as defined hereinbelow) which can be used to identify a specific polynucleotide present in samples bearing the complementary sequence.

"Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence. Thus, a "polypeptide," "protein," or "amino acid" sequence has at least about 50% identity, preferably about 60% identity, more preferably about 75-85% identity, and most preferably about 90-95% or more identity to an LU103 amino acid sequence. Further, the LU103 "polypeptide," "protein," or "amino acid" sequence may have at least about 60% similarity, preferably at least about 75% similarity, more preferably about 85% similarity, and most preferably about 95% or more similarity to a polypeptide or amino acid sequence of LU103. This amino acid sequence can be selected from the group consisting of SEQUENCE ID NOS 14-19, and fragments thereof.

A "recombinant polypeptide," "recombinant protein," or "a polypeptide produced by recombinant techniques," which terms may be used interchangeably herein, describes a polypeptide which by virtue of its origin or manipulation is not associated with all or a portion of the polypeptide with which it is associated in nature and/or is linked to a polypeptide other than that to which it is linked in nature. A recombinant or encoded polypeptide or protein is not necessarily translated from a designated nucleic acid sequence. It also may be generated in any manner, including chemical synthesis or expression of a recombinant expression system.

The term "synthetic peptide" as used herein means a polymeric form of amino acids of any length, which may be chemically synthesized by methods well-known to the routineer. These synthetic peptides are useful in various applications.

The term "polynucleotide" as used herein means a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double-

and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modifications, such as methylation or capping and unmodified forms of the polynucleotide. The terms "polynucleotide," "oligomer," "oligonucleotide," and "oligo" are used interchangeably herein.

"A sequence corresponding to a cDNA" means that the sequence contains a polynucleotide sequence that is identical or complementary to a sequence in the designated DNA. The degree (or "percent") of identity or complementarity to the cDNA will be approximately 50% or greater, preferably at least about 70% or greater, and more preferably at least about 90% or greater. The sequence that corresponds to the identified cDNA will be at least about 50 nucleotides in length, preferably at least about 60 nucleotides in length, and more preferably at least about 70 nucleotides in length. The correspondence between the gene or gene fragment of interest and the cDNA can be determined by methods known in the art and include, for example, a direct comparison of the sequenced material with the cDNAs described, or hybridization and digestion with single strand nucleases, followed by size determination of the digested fragments.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

"Purified polypeptide" or "purified protein" means a polypeptide of interest or fragment thereof which is essentially free of, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, cellular components with which the polypeptide of interest is naturally associated. Methods for purifying polypeptides of interest are known in the art.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, which is separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide

or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

"Polypeptide" and "protein" are used interchangeably herein and indicate at least one molecular chain of amino acids linked through covalent and/or non-covalent bonds. The terms do not refer to a specific length of the product. Thus peptides, oligopeptides and proteins are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide.

A "fragment" of a specified polypeptide refers to an amino acid sequence which comprises at least about 3-5 amino acids, more preferably at least about 8-10 amino acids, and even more preferably at least about 15-20 amino acids derived from the specified polypeptide.

"Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vectors or other transferred DNA, and include the original progeny of the original cell which has been transfected.

As used herein "replicon" means any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

A "vector" is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

The term "control sequence" refers to a polynucleotide sequence which is necessary to effect the expression of a coding sequence to which it is ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include a promoter, a ribosomal binding site, and terminators; in eukaryotes, such control sequences generally include promoters, terminators and, in some instances, enhancers. The term "control sequence" thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

"Operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for

example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequence.

The term "open reading frame" or "ORF" refers to a region of a polynucleotide sequence which encodes a polypeptide. This region may represent a portion of a coding sequence or a total coding sequence.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5' -terminus and a translation stop codon at the 3' -terminus. A coding sequence can include, but is not limited to, mRNA, cDNA and recombinant polynucleotide sequences.

The term "immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptide(s) which also are present in and are unique to the designated polypeptide(s). Immunological identity may be determined by antibody binding and/or competition in binding. These techniques are known to the routineer and also are described herein. The uniqueness of an epitope also can be determined by computer searches of known data banks, such as GenBank, for the polynucleotide sequence which encodes the epitope and by amino acid sequence comparisons with other known proteins.

As used herein, "epitope" means an antigenic determinant of a polypeptide or protein. Conceivably, an epitope can comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids and more usually, it consists of at least eight to ten amino acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

A "conformational epitope" is an epitope that is comprised of specific juxtaposition of amino acids in an immunologically recognizable structure, such amino acids being present on the same polypeptide in a contiguous or non-contiguous order or present on different polypeptides.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly, by the kinetics of antibody binding, and/or by competition in

binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The methods for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

As used herein, the term "immunogenic polypeptide containing an epitope of interest" means naturally occurring polypeptides of interest or fragments thereof, as well as polypeptides prepared by other means, for example, by chemical synthesis or the expression of the polypeptide in a recombinant organism.

The term "transfection" refers to the introduction of an exogenous polynucleotide into a prokaryotic or eucaryotic host cell, irrespective of the method used for the introduction. The term "transfection" refers to both stable and transient introduction of the polynucleotide, and encompasses direct uptake of polynucleotides, transformation, transduction, and f-mating. Once introduced into the host cell, the exogenous polynucleotide may be maintained as a non-integrated replicon, for example, a plasmid, or alternatively, may be integrated into the host genome.

"Treatment" refers to prophylaxis and/or therapy.

The term "individual" as used herein refers to vertebrates, particularly members of the mammalian species and includes, but is not limited to, domestic animals, sports animals, primates and humans; more particularly the term refers to humans.

The term "sense strand" or "plus strand" (or "+") as used herein denotes a nucleic acid that contains the sequence that encodes the polypeptide. The term "antisense strand" or "minus strand" (or "-") denotes a nucleic acid that contains a sequence that is complementary to that of the "plus" strand.

The term "test sample" refers to a component of an individual's body which is the source of the analyte (such as, antibodies of interest or antigens of interest). These components are well known in the art. A test sample is typically anything suspected of containing a target sequence. Test samples can be prepared using methodologies well known in the art such as by obtaining a specimen from an individual and, if necessary, disrupting any cells contained thereby to release target nucleic acids. These test samples include biological samples which can be tested by the methods of the present invention described herein and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, sputum, bronchial washing, bronchial aspirates, urine, lymph fluids and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk,

white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; tissue specimens which may be fixed; and cell specimens which may be fixed.

"Purified product" refers to a preparation of the product which has been isolated from the cellular constituents with which the product is normally associated and from other types of cells which may be present in the sample of interest.

"PNA" denotes a "peptide nucleic acid analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. "MA" denotes a "morpholino analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841. PNAs are neutrally charged moieties which can be directed against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and resistance to enzymatic degradation which is present in methods utilizing DNA or RNA. These PNAs can be labeled with ("attached to") such signal generating compounds as fluorescein, radionucleotides, chemiluminescent compounds and the like. PNAs or other nucleic acid analogs such as MAs thus can be used in assay methods in place of DNA or RNA. Although assays are described herein utilizing DNA probes, it is within the scope of the routineer that PNAs or MAs can be substituted for RNA or DNA with appropriate changes if and as needed in assay reagents.

"Analyte," as used herein, is the substance to be detected which may be present in the test sample. The analyte can be any substance for which there exists a naturally occurring specific binding member (such as, an antibody), or for which a specific binding member can be prepared. Thus, an analyte is a substance that can bind to one or more specific binding members in an assay. "Analyte" also includes any antigenic substances, haptens, antibodies and combinations thereof. As a member of a specific binding pair, the analyte can be detected by means of naturally occurring specific binding partners (pairs) such as the use of intrinsic factor protein as a member of a specific binding pair for the determination of Vitamin B12, the use of folate-binding protein to determine folic acid, or the use of a lectin as a member of a specific binding pair for the determination of a carbohydrate. The analyte can include a protein, a polypeptide, an amino acid, a nucleotide target and the like.

"Diseases of the lung," or "lung disease" or "condition of the lung," as used herein, refer to any disease or condition of the l respiratory tract including, but not limited to, pneumonia (of all origins, inclu iral, bacterial, and fungal), asthma, black lung disease, silicosis, adult resp ry distress syndrome, and cancer.

"Lung cancer," as used herein, refers to malignant disease of the lower respiratory tract including, but not limited to, sm ell carcinoma, adenocarcinoma, squamous cell carcinoma, and large cell carcino Lung cancers are frequently grouped into small cell carcinoma and non-small ell carcinomas.

An "Expressed Sequence Tag" or "EST" fers to the partial sequence of a cDNA insert which has been made by reverse tr scription of mRNA extracted from a tissue followed by insertion into a vector.

A "transcript image" refers to a table or l giving the quantitative distribution of ESTs in a library and represents the genes act e in the tissue from which the library was made.

The present invention provides assays w h utilize specific binding members. A "specific binding member," as use erein, is a member of a specific binding pair. That is, two different molecules w ere one of the molecules, through chemical or physical means, specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes and the like. Furthermore, specific binding pairs can include members that are analo s of the original specific binding members, for example, an analyte-analog. Imm reactive specific binding members include antigens, antigen fragments, a bodies and antibody fragments, both monoclonal and polyclonal and complexes ereof, including those formed by recombinant DNA molecules.

The term "hapten," as used herein, refer a partial antigen or non-protein binding member which is capable of binding to antibody, but which is not capable of eliciting antibody formation unless coupled t carrier protein.

A "capture reagent," as used herein, refe to an unlabeled specific binding member which is specific either for the analyte n a sandwich assay, for the indicator reagent or analyte as in a competitive a y, or for an ancillary specific binding member, which itself is specific for the alyte, as in an indirect assay. The

capture reagent can be directly or indirectly bound to a solid phase material before the performance of the assay or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample.

The "indicator reagent" comprises a "signal-generating compound" ("label") which is capable of generating and generates a measurable signal detectable by external means, conjugated ("attached") to a specific binding member. In addition to being an antibody member of a specific binding pair, the indicator reagent also can be a member of any specific binding pair, including either hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to the polypeptide of interest as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay. When describing probes and probe assays, the term "reporter molecule" may be used. A reporter molecule comprises a signal generating compound as described hereinabove conjugated to a specific binding member of a specific binding pair, such as carbazole or adamantane.

The various "signal-generating compounds" (labels) contemplated include chromagens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums and luminol, radioactive elements and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase and the like. The selection of a particular label is not critical, but it must be capable of producing a signal either by itself or in conjunction with one or more additional substances.

"Solid phases" ("solid supports") are known to those in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic or non-magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells and Duracytes® (red blood cells "fixed" by pyruvic aldehyde and formaldehyde, available from Abbott Laboratories, Abbott Park, IL) and others. The "solid phase" is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon

chips, sheep (or other suitable animal's) red blood cells and Duracytes® are all suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase," as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, Duracytes® and other configurations known to those of ordinary skill in the art.

It is contemplated and within the scope of the present invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structures generally are preferred, but materials with a gel structure in the hydrated state may be used as well. Such useful solid supports include, but are not limited to, nitrocellulose and nylon. It is contemplated that such porous solid supports described herein preferably are in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1 mm. The pore size may vary within wide limits and preferably is from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surface of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Other suitable solid supports are known in the art.

Reagents

The present invention provides reagents such as polynucleotide sequences derived from a lung tissue of interest and designated as LU103, polypeptides

encoded thereby and antibodies specific for these polypeptides. The present invention also provides reagents such as oligonucleotide fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides. The polynucleotides, polypeptides, or antibodies of the present invention may be used to provide information leading to the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating of, or determining the predisposition to, diseases and conditions of the lung, such as lung cancer. The sequences disclosed herein represent unique polynucleotides which can be used in assays or for producing a specific profile of gene transcription activity. Such assays are disclosed in European Patent Number 0373203B1 and International Publication No. WO 95/11995.

Selected LU103-derived polynucleotides can be used in the methods described herein for the detection of normal or altered gene expression. Such methods may employ LU103 polynucleotides or oligonucleotides, fragments or derivatives thereof, or nucleic acid sequences complementary thereto.

The polynucleotides disclosed herein, their complementary sequences, or fragments of either, can be used in assays to detect, amplify or quantify genes, nucleic acids, cDNAs or mRNAs relating to lung tissue disease and conditions associated therewith. They also can be used to identify an entire or partial coding region of an LU103 polypeptide. They further can be provided in individual containers in the form of a kit for assays, or provided as individual compositions. If provided in a kit for assays, other suitable reagents such as buffers, conjugates and the like may be included.

The polynucleotide may be in the form of RNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, nucleic acid analogs and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded, may be the coding (sense) strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA provided herein.

This polynucleotide may include only the coding sequence for the polypeptide, or the coding sequence for the polypeptide and an additional coding sequence such as a leader or secretory sequence or a proprotein sequence, or the coding sequence for the polypeptide (and optionally an additional coding sequence)

and a non-coding sequence, such as a non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide.

In addition, the invention includes variant polynucleotides containing modifications such as polynucleotide deletions, substitutions or additions; and any polypeptide modification resulting from the variant polynucleotide sequence. A polynucleotide of the present invention also may have a coding sequence which is a naturally occurring allelic variant of the coding sequence provided herein.

In addition, the coding sequence for the polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the polypeptide. The polynucleotides may also encode for a proprotein which is the protein plus additional 5' amino acid residues. A protein having a prosequence is a proprotein and may, in some cases, be an inactive form of the protein. Once the prosequence is cleaved an active protein remains. Thus, the polynucleotide of the present invention may encode for a protein, or for a protein having a prosequence, or for a protein having both a presequence (leader sequence) and a prosequence.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. a COS-7 cell line, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein. See, for example, I. Wilson et al., Cell 37:767 (1984).

It is contemplated that polynucleotides will be considered to hybridize to the sequences provided herein if there is at least 50%, preferably at least 70%, and more preferably at least 90% identity between the polynucleotide and the sequence.

The present invention also provides an antibody produced by using a purified LU103 polypeptide of which at least a portion of the polypeptide is encoded by an LU103 polynucleotide selected from the polynucleotides provided herein. These antibodies may be used in the methods provided herein for the detection of LU103 antigen in test samples. The presence of LU103 antigen in the test samples is

indicative of the presence of a lung dise condition. The antibody also may be used for therapeutic purposes, for exam n neutralizing the activity of LU103 polypeptide in conditions associated wi ered or abnormal expression.

The present invention further re to an LU103 polypeptide which has the deduced amino acid sequence as provid rein, as well as fragments, analogs and derivatives of such polypeptide. The pol ptide of the present invention may be a recombinant polypeptide, a natural purif polypeptide or a synthetic polypeptide. The fragment, derivative or analog of the U103 polypeptide may be one in which one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a le r or secretory sequence or a sequence which is employed for purification of the lypeptide or a proprotein sequence. Such fragments, derivatives and analogs are within the scope of the present invention. The polypeptides and polynucleotides of the present invention are provided preferably in an isolated form and preferably purified.

Thus, a polypeptide of the present invention may have an amino acid sequence that is identical to that of the naturally occurring polypeptide or that is different by minor variations due to one or more amino acid substitutions. The variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine or threonine with serine. In contrast, variations may include nonconservative changes, e.g., replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without changing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR Inc., Madison WI).

Probes constructed according to polynucleotide sequences of the present invention can be used in various assay methods to provide various types of analysis.

For example, such probes can be used in fluorescent in situ hybridization (FISH) technology to perform chromosomal analysis, and used to identify cancer-specific structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR-generated and/or allele specific oligonucleotides probes, allele specific amplification or by direct sequencing. Probes also can be labeled with radioisotopes, directly- or indirectly-detectable haptens, or fluorescent molecules, and utilized for in situ hybridization studies to evaluate the mRNA expression of the gene comprising the polynucleotide in tissue specimens or cells.

This invention also provides teachings as to the production of the polynucleotides and polypeptides provided herein.

Probe Assays

The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of the routineer. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multi-gene family or in related species like mouse and man.

The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers are employed in excess to hybridize to the complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New primers then are hybridized and extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in U.S. Patent Nos 4,683,195 and 4,683,202.

The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess to target. The first probe hybridizes to a first segment of the target

strand, and the second probe hybridizes to a second segment of the target strand, first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3' hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more completely in EP-A- 320 308, published June 16, 1989, and EP-A-439 182, published July 31, 1991.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770; or reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R.L. Marshall et al., PCR Methods and Applications 4:80-84 (1994).

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described by J.C Guatelli, et al., PNAS USA 87:1874-1878 (1990) and also described by J. Compton, Nature 350 (No. 6313):91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G.T. Walker et al., Clin. Chem. 42:9-13 (1996)) and European Patent Application No. 684315; and target mediated amplification, as described in International Publication No. WO 93/22461.

Detection of LU103 may be accomplished using any suitable detection method, including those detection methods which are currently well known in the art, as well as detection strategies which may evolve later. See, for example, Caskey et al., and U.S. Patent Nos 5,582,989 and 5,210,015. Examples of such detection methods include target amplification methods as well as signal amplification technologies. An example of presently known detection methods would include the

nucleic acid amplification technologies re ·red to as PCR, LCR, NASBA, SDA, RCR and TMA. See, for example, U.S. ·ent No. 5,582,989 to Caskey et al., and U.S. Patent No. 5,210,015 Gelfand et al. ·tection may also be accomplished using signal amplification such as that di ·losed in U.S. Patent No. 5,273,882 to Snitman et al.. While the amplification o: ·rget or signal is preferred at present, it is contemplated and within the scope of the ·esent invention that ultrasensitive detection methods which do not require ar ·lification can be utilized herein.

Detection, both amplified and non ·mplified, may be (combined) carried out using a variety of heterogeneous and hom ·geneous detection formats. Examples of heterogeneous detection formats are disc·osed in U.S. Patent No. 5,273,882 to Snitman et al., EP-84114441.9, U.S. Pat·nt No. 5,124,246 to Urdea et al., U.S. Patent No. 5,185,243 to Ullman et al. an·l U.S. Patent No. 4,581,333 to Kourilsky et al.. Examples of homogeneous detecti·n formats are disclosed in U.S. Patent No. 5,582,989 to Caskey et al., and U.S. Patent No. 5,210,015 to Gelfand et al. Also contemplated and within the scope o ·he present invention is the use of multiple probes in the hybridization assay which use improves sensitivity and amplification of the LU103 signal. See, ·r example, U.S. Patent Nos. 5,582,989 and 5,210,015.

In one embodiment, the present in·ention generally comprises the steps of contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method provided herein are labeled with capture and detection labels, whereir probes are labeled with one type of label and primers are labeled with another type ·f label. Additionally, the primers and probes are selected such that the probe se· uence has a lower melt temperature than the primer sequences. The amplification ·eagents, detection reagents and test sample are placed under amplification conditions whereby, in the presence of target sequence, copies of the target sequence (a·1 amplicon) are produced. In the usual case, the amplicon is double stranded bec ·use primers are provided to amplify a target sequence and its complementary st· ·nd. The double stranded amplicon then is thermally denatured to produce single str· ·ded amplicon members. Upon formation of the single stranded amplicon members. ·he mixture is cooled to allow the formation of complexes between the prob ·s and single stranded amplicon members.

As the single stranded amplicon sequences and probe sequences are cooled, th · probe sequences preferentially bind the single stranded amplicon members. This fi..ing is counterintuitive given that the probe sequences generally are selected to be sh orter than the primer sequences and therefore have a lower melt temperature than th : primers. Accordingly, the melt temperature of the amplicon produced by the primers should also have a higher melt temperature than the probes. Thus, as the mixture cools, the re-formation of the double stranded amplicon would be expected. As previously stated, however, this is not the case. The probes are found to preferentially bind the single stranded amplicon members. Moreover, this preference of probe/single stranded amplicon binding exists even when the primer sequences are added in excess of the probes.

After the probe/single stranded amplicon member hybrids are formed, they are detected. Standard heterogeneous assay formats are suitable for detecting the hybrids using the detection labels and capture labels present on the primers and probes. The hybrids can be bound to a solid phase reagent by virtue of the capture label and detected by virtue of the detection label. In cases where the detection label is directly detectable, the presence of the hybrids on the solid phase can be detected by causing the label to produce a detectable signal, if necessary, and detecting the signal. In cases where the label is not directly detectable, the captured hybrids can be contacted with a conjugate, which generally comprises a binding member attached to a directly detectable label. The conjugate becomes bound to the complexes and the conjugates presence on the complexes can be detected with the directly detectable label. Thus, the presence of the hybrids on the solid phase reagent can be determined. Those skilled in the art will recognize that wash steps may be employed to wash away unhybridized amplicon or probe as well as unbound conjugate.

Although the target sequence is described as single stranded, it also is contemplated to include the case where the target sequence is actually double stranded but is merely separated from its complement prior to hybridization with the amplification primer sequences. In the case where PCR is employed in this method, the ends of the target sequences are usually known. In cases where LCR or a modification thereof is employed in the preferred method, the entire target sequence is usually known. Typically, the target sequence is a nucleic acid sequence such as, for example, RNA or DNA.

The method provided herein can be used in well-known amplification reactions that include thermal cycle reaction mixtures, particularly in PCR and gap

LCR (GLCR). Amplification reactions typically employ primers to repeatedly generate copies of a target nucleic acid sequence, which target sequence is usually a small region of a much larger nucleic acid sequence. Primers are themselves nucleic acid sequences that are complementary to regions of a target sequence. Under amplification conditions, these primers hybridize or bind to the complementary regions of the target sequence. Copies of the target sequence typically are generated by the process of primer extension and/or ligation which utilizes enzymes with polymerase or ligase activity, separately or in combination, to add nucleotides to the hybridized primers and/or ligate adjacent probe pairs. The nucleotides that are added to the primers or probes, as monomers or preformed oligomers, are also complementary to the target sequence. Once the primers or probes have been sufficiently extended and/or ligated, they are separated from the target sequence, for example, by heating the reaction mixture to a "melt temperature" which is one in which complementary nucleic acid strands dissociate. Thus, a sequence complementary to the target sequence is formed.

A new amplification cycle then can take place to further amplify the number of target sequences by separating any double stranded sequences, allowing primers or probes to hybridize to their respective targets, extending and/or ligating the hybridized primers or probes and re-separating. The complementary sequences that are generated by amplification cycles can serve as templates for primer extension or filling the gap of two probes to further amplify the number of target sequences. Typically, a reaction mixture is cycled between 20 and 100 times, more typically, a reaction mixture is cycled between 25 and 50 times. The numbers of cycles can be determined by the routineer. In this manner, multiple copies of the target sequence and its complementary sequence are produced. Thus, primers initiate amplification of the target sequence when it is present under amplification conditions.

Generally, two primers which are complementary to a portion of a target strand and its complement are employed in PCR. For LCR, four probes, two of which are complementary to a target sequence and two of which are similarly complementary to the target's complement, are generally employed. In addition to the primer sets and enzymes previously mentioned, a nucleic acid amplification reaction mixture may also comprise other reagents which are well known and include but are not limited to: enzyme cofactors such as manganese; magnesium; salts; nicotinamide adenine dinucleotide (NAD); and deoxynucleotide triphosphates

(dNTPs) such, as for example, deoxyadenine t hate, deoxyguanine triphosphate, deoxycytosine triphosphate and t ymine triphosphate.

While the amplification primers initiate ..i cation of the target sequence, the detection (or hybridization) probe is not inv d n amplification. Detection probes are generally nucleic acid sequences or ed nucleic acid analogs such as, for example, peptide nucleic acids which ar e sed in International Publication No. WO 92/20702; morpholino an which are described in U.S. Patents Nos 5,185,444, 5,034,506 and 5,142,0 and the like. Depending upon the type of label carried by the probe, the probe is employed to capture or detect the amplicon generated by the amplification reaction The probe is not involved in amplification of the target sequence and therefore may have to be rendered "non-extendible" in that additional dNTPs cannot be added to the probe. In and of themselves analogs usually are non-extendible and nucleic acid probes can be rendered non-extendible by modifying the 3' end of the probe such that the hydroxyl group is no longer capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby consume or otherwise block the hydroxyl grou . Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified. U.S. Patent Application Serial No. 07/049,061 filed April 19, 1993 describes modifications which can be used to render a probe non-extendible.

The ratio of primers to probes is not important. Thus, either the probes or primers can be added to the reaction mixture in excess whereby the concentration of one would be greater than the concentration of the other. Alternatively, primers and probes can be employed in equivalent concentrations. Preferably, however, the primers are added to the reaction mixture in excess of the probes. Thus, primer to probe ratios of, for example, 5:1 and 20:1 are preferred.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences. Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the rang between 20 and 50 nucleotides long, more typically in the range of between 2 0 nucleotides long. The typical probe is in the range of between 10 and 25 nucl tides long.

Various methods for synthesizing prim and probes are well known in the art. Similarly, methods for attaching labels to p ne or probes are also well known in the art. For example, it is a matter of routine to synthesize desired nucleic acid

primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), DuPont (Wilmington, DE), or Milligen (Bedford MA). Many methods have been described for labeling oligonucleotides such as the primers or probes of the present invention. Enzo Biochemical (New York, NY) and Clontech (Palo Alto, CA) both have described and commercialized probe labeling techniques. For example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (Clontech, Palo Alto, CA). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (Clontech). The amines can be reacted to various haptens using conventional activation and linking chemistries. In addition, copending applications U.S. Serial Nos. 625,566, filed December 11, 1990, and 630,908, filed December 20, 1990, teach methods for labeling probes at their 5' and 3' termini, respectively. International Publication Nos WO 92/10505, published 25 June 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling probes at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J.S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' and 5' ends.

A capture label is attached to the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

Generally, probe/single stranded amplicon member complexes can be detected using techniques commonly employed to perform heterogeneous immunoassays. Preferably, in this embodiment, detection is performed according to

the protocols used by the commercially available Abbott LCx® instrumentation (Abbott Laboratories, Abbott Park, IL).

The primers and probes disclosed herein are useful in typical PCR assays, wherein the test sample is contacted with a pair of primers, amplification is performed, the hybridization probe is added, and detection is performed.

Another method provided by the present invention comprises contacting a test sample with a plurality of polynucleotides, wherein at least one polynucleotide is an LU103 molecule as described herein, hybridizing the test sample with the plurality of polynucleotides and detecting hybridization complexes. Hybridization complexes are identified and quantitated to compile a profile which is indicative of lung tissue disease, such as lung cancer. Expressed RNA sequences may further be detected by reverse transcription and amplification of the DNA product by procedures well-known in the art, including polymerase chain reaction (PCR).

Drug Screening and Gene Therapy.

The present invention also encompasses the use of gene therapy methods for the introduction of anti-sense LU103 derived molecules, such as polynucleotides or oligonucleotides of the present invention, into patients with conditions associated with abnormal expression of polynucleotides related to a lung tissue disease or condition, especially lung cancer. These molecules, including antisense RNA and DNA fragments and ribozymes, are designed to inhibit the translation of LU103-mRNA, and may be used therapeutically in the treatment of conditions associated with altered or abnormal expression of an LU103 polynucleotide.

Alternatively, the oligonucleotides described above can be delivered to cells by procedures known in the art such that the anti-sense RNA or DNA may be expressed in vivo to inhibit production of an LU103 polypeptide in the manner described above. Antisense constructs to an LU103 polynucleotide, therefore, reverse the action of LU103 transcripts and may be used for treating lung tissue disease conditions, such as lung cancer. These antisense constructs may also be used to treat tumor metastases.

The present invention also provides a method of screening a plurality of compounds for specific binding to LU103 polypeptide(s), or any fragment thereof, to identify at least one compound which specifically binds the LU103 polypeptide. Such a method comprises the steps of providing at least one compound; combining the LU103 polypeptide with each compound under suitable conditions for a time

sufficient to allow binding; and detecting the LU103 polypeptide binding to each compound.

The polypeptide or peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of screening utilizes eukaryotic or prokaryotic host cells which are stably transfected with recombinant nucleic acids which can express the polypeptide or peptide fragment. A drug, compound, or any other agent, may be screened against such transfected cells in competitive binding assays. For example, the formation of complexes between a polypeptide and the agent being tested can be measured in either viable or fixed cells.

The present invention thus provides methods of screening for drugs, compounds, or any other agent which can be used to treat diseases associated with LU103. These methods comprise contacting the agent with a polypeptide or fragment thereof and assaying for either the presence of a complex between the agent and the polypeptide, or for the presence of a complex between the polypeptide and the cell. In competitive binding assays, the polypeptide typically is labeled. After suitable incubation, free (or uncomplexed) polypeptide or fragment thereof is separated from that present in bound form, and the amount of free or uncomplexed label is used as a measure of the ability of the particular agent to bind to the polypeptide or to interfere with the polypeptide/cell complex.

The present invention also encompasses the use of competitive screening assays in which neutralizing antibodies capable of binding polypeptide specifically compete with a test agent for binding to the polypeptide or fragment thereof. In this manner, the antibodies can be used to detect the presence of any polypeptide in the test sample which shares one or more antigenic determinants with an LU103 polypeptide as provided herein.

Another technique for screening provides high throughput screening for compounds having suitable binding affinity to at least one polypeptide of LU103 disclosed herein. Briefly, large numbers of different small peptide test compounds are synthesized on a solid phase, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptide and washed. Polypeptide thus bound to the solid phase is detected by methods well-known in the art. Purified polypeptide can also be coated directly onto plates for use in the screening techniques described herein. In addition, non-neutralizing antibodies can be used to capture the

pol eptide a immobilize it on the solid support. See, for example, EP 84/03564, pul shed on September 13, 1984.

The goal of rational drug design is to produce structural analogs of bio gically active polypeptides of interest or of the small molecules including ago ists, anta onists, or inhibitors with which they interact. Such structural analogs can be used to design drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide in vivo. J. Hodgson, Bio/Technology 9:19-21 (1991).

For example, in one approach, the three-dimensional structure of a polypeptide, or of a polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous polypeptide-like molecules or to identify efficient inhibitors

Useful examples of rational drug design may include molecules which have improved activity or stability as shown by S. Braxton et al., Biochemistry 31:7796-7801 (1992), or which act as inhibitors, agonists, or antagonists of native peptides as shown by S.B.P. Athauda et al., J. Biochem. (Tokyo) 113 (6):742-746 (1993).

It also is possible to isolate a target-specific antibody selected by an assay as described hereinabove, and then to determine its crystal structure. In principle, this approach yields a pharmacophore upon which subsequent drug design can be based. It further is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies ("anti-ids") to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-id is an analog of the original receptor. The anti-id then can be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides then can act as the pharmacophore (that is, a prototype pharmaceutical drug).

A sufficient amount of a recombinant polypeptide of the present invention may be made available to perform analytical studies such as X-ray crystallography. In addition, knowledge of the polypeptide amino acid sequence which is derivable from the nucleic acid sequence provided herein will provide guidance to those

employing computer modeling techniques in place of, or in addition to, x-ray crystallography.

Antibodies specific to an LU103 polypeptide (e.g., anti-LU103 antibodies) further may be used to inhibit the biological action of the polypeptide by binding to the polypeptide. In this manner, the antibodies may be used in therapy, for example, to treat lung tissue diseases, including lung cancer and its metastases.

Further, such antibodies can detect the presence or absence of the LU103 polypeptide in a test sample and, therefore, are useful as diagnostic markers for the diagnosis of a lung tissue disease or condition, especially lung cancer. Such antibodies may also function as a diagnostic marker for lung tissue disease conditions, such as lung cancer. The present invention also is directed to antagonists and inhibitors of the polypeptides of the present invention. The antagonists and inhibitors are those which inhibit or eliminate the function of the polypeptide. Thus, for example, an antagonist may bind to a polypeptide of the present invention and inhibit or eliminate its function. The antagonist, for example, could be an antibody against the polypeptide which eliminates the activity of the LU103 polypeptide by binding the LU103 polypeptide, or in some cases the antagonist may be an oligonucleotide. Examples of small molecule inhibitors include, but are not limited to, small peptides or peptide-like molecules.

The antagonists and inhibitors may be employed as a composition with a pharmaceutically acceptable carrier, including, but not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. Administration of LU103 polypeptide inhibitors is preferably systemic. The present invention also provides an antibody which inhibits the action of such a polypeptide.

Antisense technology can be used to reduce gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the polypeptide of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription, thereby preventing transcription and the production of the LU103 polypeptide. For triple helix, see, for example, Lee et al, Nuc. Acids Res. 6:3073 (1979); Cooney et al, Science 241:456 (1988); and Dervan et al, Science 251:1360 (1991) The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of a mRNA molecule into the LU103

polypeptide. For antisense, see, for example, (, J. Neurochem. 56:560 (1991); and "Oligodeoxynucleotides as Antisen hibitors of Gene Expression," CRC Press, Boca Raton, Fla. (1988). Antisens gonucleotides act with greater efficacy when modified to contain artificial inte kotide linkages which render the molecule resistant to nucleolytic cleavage. Suc cial internucleotide linkages include, but are not limited to, methylphosphon phosphorothiolate and phosphoroamydate internucleotide linkages.

Recombinant Technology.

The present invention provides host cell d expression vectors comprising LU103 polynucleotides of the present invention d methods for the production of the polypeptide(s) they encode. Such methods c mprise culturing the host cells under conditions suitable for the expression of t LU103 polynucleotide and recovering the LU103 polypeptide from the cell lture.

The present invention also provides vec which include LU103 polynucleotides of the present invention, host ce which are genetically engineered with vectors of the present invention and the pr tion of polypeptides of the present invention by recombinant techniques.

Host cells are genetically engineered (tr fected, transduced or transformed) with the vectors of this invention which may be cloning vectors or expression vectors. The vector may be in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transfected cells, or amplifying LU103 gene(s). The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected r expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present inver n may be employed for producing a polypeptide by recombinant techniques. Thus the polynucleotide sequence may be included in any one of a variety of expressio vehicles, in particular vectors or plasmids for expressing a polypeptide. Such ve rs include chromosomal, nonchromosomal and synthetic DNA sequence g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors d ved from combinations of plasmids and phage DNA, viral DNA such as v inia, adenovirus, fowl pox virus and pseudorabies. However, any other plasmid vector may be used so long as it is replicable and viable in the host.

appropriate DNA sequence may be inserted into the vector by a variety of pro ces. In general, the DNA sequence is inserted into appropriate restriction endor ase sites by procedures known in the art. Such procedures and others are deeme be within the scope of those skilled in the art. The DNA sequence in the expre vector is operatively linked to an appropriate expression control seque s) (promoter) to direct mRNA synthesis. Representative examples of such prome include, but are not limited to, the LTR or the SV40 promoter, the E. coli lac or trp, the phage lambda P sub L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of transfected host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transfect an appropriate host to permit the host to express the protein. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Salmonella typhimurium; Streptomyces sp.; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings provided herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available. The following vectors are provided by way of example. Bacterial: pINCY (Incyte Pharmaceuticals Inc., Palo Alto, CA), pSPORT1 (Life Technologies, Gaithersburg, MD), pQE70, pQE60, pQE-9 (Qiagen) pBs, phagescript, psiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5

(Pharmacia); Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

Plasmid pINCY is generally identical to the plasmid pSPORT1 (available from Life Technologies, Gaithersburg, MD) with the exception that it has two modifications in the polylinker (multiple cloning site). These modifications are (1) it lacks a HindIII restriction site and (2) its EcoRI restriction site lies at a different location. pINCY is created from pSPORT1 by cleaving pSPORT1 with both HindIII and EcoRI and replacing the excised fragment of the polylinker with synthetic DNA fragments (SEQUENCE ID NO 6 and SEQUENCE ID NO 7). This replacement may be made in any manner known to those of ordinary skill in the art. For example, the two nucleotide sequences, SEQUENCE ID NO 6 and SEQUENCE ID NO 7, may be generated synthetically with 5' terminal phosphates, mixed together, and then ligated under standard conditions for performing staggered end ligations into the pSPORT1 plasmid cut with HindIII and EcoRI. Suitable host cells (such as E. coli DH5∝ cells) then are transfected with the ligated DNA and recombinant clones are selected for ampicillin resistance. Plasmid DNA then is prepared from individual clones and subjected to restriction enzyme analysis or DNA sequencing in order to confirm the presence of insert sequences in the proper orientation. Other cloning strategies known to the ordinary artisan also may be employed.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, SP6, T7, gpt, lambda P sub R, P sub L and trp. Eukaryotic promoters include cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, LTRs from retroviruses and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention provides host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (L. Davis et al., "Basic Methods in Molecular

Biology," 2nd edition, Appleton and Lang, Pare Publishing, East Norwalk, CT (1994)).

The constructs in host cells can be used i ivventional manner to produce the gene product encoded by the recombinant se . Alternatively, the polypeptides of the invention can be synthetically iced by conventional peptide synthesizers.

Recombinant proteins can be expressed i mmalian cells, yeast, bacteria, or other cells, under the control of appropriate pr ers. Cell-free translation systems can also be employed to produce such p ns using RNAs derived from the DNA constructs of the present invention. App riate cloning and expression vectors for use with prokaryotic and eukaryotic hos s are described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Sec d Edition, (Cold Spring Harbor, N.Y., 1989).

Transcription of a DNA encoding the pol tide(s) of the present invention by higher eukaryotes is increased by inserting an ncer sequence into the vector. Enhancers are cis-acting elements of DNA, usua out from 10 to 300 bp, that act on a promoter to increase its transcription. Exam include the SV40 enhancer on the late side of the replication origin (bp 100 to 270 , a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late ide of the replication origin and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transfection of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons enc ng glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), alpha factor, d phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing cretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial u constructed by inserting a structural DNA sequence encoding a desired pro together with suitable translation

initiat[illegible] termination signals in operable reading phase with a functional promo[illegible]e vector will comprise one or more phenotypic selectable markers and an ori[illegible] replication to ensure maintenance of the vector and to, if desirable, provid[illegible]lification within the host. Suitable prokaryotic hosts for transfection includ[illegible]li, Bacillus subtilis, Salmonella typhimurium and various species within the gen[illegible] Pseudomonas, Streptomyces and Staphylococcus, although, others may also be employed as a routine matter of choice.

Useful expression vectors for bacterial use comprise a selectable marker and bacteri[illegible] origin of replication derived from plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Other vectors include but are not limited to PKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transfection of a suitable host and growth of the host to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction), and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents; such methods are well-known to the ordinary artisan.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, such as the C127, HEK-293, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Representative, useful vectors include pRC/CMV and pcDNA3 (available from Invitrogen, San Diego, CA).

LU103 polypeptides are recovered and purified from recombinant cell cultures by known methods including affinity chromatography, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. It is preferred to have low concentrations (approximately 0.1-5 mM) of calcium ion present during purification (Price, et al., J. Biol. Chem. 244:917 (1969)). Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Thus, polypeptides of the present invention may be naturally purified products expressed from a high expressing cell line, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. The polypeptides of the invention may also include an initial methionine amino acid residue.

The starting plasmids can be constructed from available plasmids in accord with published, known procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

The following is the general procedure for the isolation and analysis of cDNA clones. In a particular embodiment disclosed herein, mRNA was isolated from lung tissue and used to generate the cDNA library. Lung tissue was obtained from patients by surgical resection and was classified as tumor or non-tumor tissue by a pathologist.

The cDNA inserts from random isolates of the lung tissue libraries were sequenced in part, analyzed in detail as set forth in the Examples and are disclosed in the Sequence Listing as SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3. The consensus sequence of these inserts is presented as SEQUENCE ID NO 4. These polynucleotides may contain an entire open reading frame with or without associated regulatory sequences for a particular gene, or they may encode only a portion of the gene of interest. This is attributed to the fact that many genes are several hundred and sometimes several thousand bases in length

art, with current technology, cannot be cloned in the society because of vector limitations, incomplete reverse transcription of the first, or incomplete replication of the second strand. Contiguous, second clones containing additional nucleotide sequences may be obtained using a variety of methods known to those of skill in the art.

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ DNA polymerase, Klenow fragment, Sequenase (US Biochemical Corp., Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single-stranded and double-stranded templates. The chain termination reaction products may be electrophoresed on urea/polyacrylamide gels and detected either by autoradiography (for radionucleotide labeled precursors) or by fluorescence (for fluorescence-labeled precursors). Recent improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day using machines such as Applied Biosystems 377 DNA Sequencers (Applied Biosystems, Foster City

The reading frame of the nucleotide sequence can be ascertained by several types of analyses. First, reading frames contained within the coding sequence can be analyzed for the presence of start codon ATG and stop codons TGA, TAA or TAG. Typically, one reading frame will continue throughout the major portion of a cDNA sequence while other reading frames tend to contain numerous stop codons. In such cases, reading frame determination is straightforward. In other more difficult cases, further analysis is required.

Algorithms have been created to analyze the occurrence of individual nucleotide bases at each putative codon triplet. See, for example J.W. Fickett, Nuc Acids Res 10:5303 (1982). Coding DNA for particular organisms (bacteria, plants and animals) tends to contain certain nucleotides with certain triplet periodicities, such as a significant preference for pyrimidines in third codon position. These preferences have been incorporated into widely available software which can be used to determine coding potential (and frame) of a given stretch of DNA. The algorithm-derived information combined with start/stop codon information can be used to determine proper frame with a high degree of certainty. This, in turn, readily permits cloning of the sequence in the correct reading frame into appropriate expression vectors.

The ...cid sequences disclosed herein may be joined to a variety o... other polyn... sequences and vectors of interest by means of well-establi... recombinan... ...echniques. See J. Sambrook et al., supra. Vectors of inter... include clon... ...ors, such as plasmids, cosmids, phage derivatives, phagen... as well as sc... ...g, replication and expression vectors, and the like. In gene... such vectors ...n an origin of replication functional in at least one organism, convenient r... ...on endonuclease digestion sites and selectable markers appropriate f... ...articular host cells. The vectors can be transferred by a variety o... means known ... those of skill in the art into suitable host cells which then prod...ce the desired DNA, RNA or polypeptides.

Occasionally, sequencing or random reverse transcription errors will mask the presence of the appropriate open reading frame or regulatory element. In such cases, it is possible to determine the correct reading frame by attempting to express the polypeptide and determining the amino acid sequence by standard peptide mapping and ...uencing techniques. See, F.M. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY (1989). Additionally the actual reading ...me of a given nucleotide sequence may be determined by transfection of host cells with vectors containing all three potential reading frames. Only those cells with the nucleotide sequence in the correct reading frame will produce a peptide of the predicted length.

The nucleotide sequences provided herein have been prepared by current, state-of-the-art, automated methods and as such may contain unidentified nucleotides. These will not present a problem to those skilled in the art who wish to practice the invention. Several methods employing standard recombinant techniques, described in J. Sambrook (supra) or periodic updates thereof, may be used to complete the missing sequence information. The same techniques used for obtaining a full length sequence, as described herein, may be used to obtain nucleotide sequences.

Expr...ion of a particular cDNA may be accomplished by subcloning the cDNA into an appropriate expression vector and transfecting this vector into an appropriate e...ression host. The cloning vector used for the generation of the ...ng tissue cDNA library can be used for transcribing mRNA of a particular cDNA and contains a pr...oter for beta-galactosidase, an amino-terminal met and the subsequent s... amino acid residues of beta-galactosidase. Immediately follo...ng these eight residues is an engineered bacteriophage promoter, useful for artificial

priming and transcription, as well as a number of unique restriction sites, including EcoRI, for cloning. The vector can be transfected into an appropriate host strain of E. coli.

Induction of the isolated bacterial strain with isopropylthiogalactoside (IPTG) using standard methods will produce a fusion protein which contains the first seven residues of beta-galactosidase, about 15 residues of linker and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, the correct frame can be obtained by deletion or insertion of an appropriate number of bases by well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide primers, containing cloning sites and segments of DNA sufficient to hybridize to stretches at both ends of the target cDNA, can be synthesized chemically by standard methods. These primers can then be used to amplify the desired gene segments by PCR. The resulting new gene segments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments can be produced by digestion of the cDNA with appropriate restriction enzymes and filling in the missing gene segments with chemically synthesized oligonucleotides. Segments of the coding sequence from more than one gene can be ligated together and cloned in appropriate vectors to optimize expression of recombinant sequence.

Suitable expression hosts for such chimeric molecules include but are not limited to, mammalian cells such as Chinese Hamster Ovary (CHO) and human embryonic kidney (HEK) 293 cells, insect cells such as Sf9 cells, yeast cells such as Saccharomyces cerevisiae and bacteria such as E. coli. For each of these cell systems, a useful expression vector may also include an origin of replication to allow propagation in bacteria and a selectable marker such as the beta-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors may include a second selectable marker, such as the neomycin phosphotransferase gene, to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts may require the addition of 3' poly A tail if the sequence of interest lacks poly A.

Additionally, the vector may contain promoters ... cers which increase gene expression. Such promoters are host specific and ... but are not limited to, MMTV, SV40, or metallothionine promoters for CI ... trp, lac, tac or T7 promoters for bacterial hosts; or alpha factor, alcohol o ... or PGH promoters for yeast. Adenoviral vectors with or without transcriptior ... ers, such as the rous sarcoma virus (RSV) enhancer, may be used to drive pr ... xpression in mammalian cell lines. Once homogeneous cultures of r ... mant cells are obtained, large quantities of recombinantly produced pr ... can be recovered from the conditioned medium and analyzed using chromatogra ... c methods well known in the art. An alternative method for the production of l ... amounts of secreted protein involves the transfection of mammalian embryos ... d the recovery of the recombinant protein from milk produced by transgenic c ... ws, goats, sheep, etc. Polypeptides and closely related molecules may be expre ... ed recombinantly in such a way as to facilitate protein purification. One approach ... olves expression of a chimeric protein which includes one or more additional ... peptide domains not naturally present on human polypeptides. Such purifica ... facilitating domains include, but are not limited to, metal-chelating peptides ... as histidine-tryptophan domains that allow purification on immobilized metals, p ... tein A domains that allow purification on immobilized immunoglobulin and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase from Invitrogen (San Diego, CA) between the polypeptide sequence and the purification domain may be useful for recovering the polypeptide.

Immunoassays

LU103 polypeptides, including fragments, deriva ... es, and analogs thereof, or cells expressing such polypeptides, can be utilized in ... variety of assays, many of which are described herein, for the detection of antibodi ... to lung tissue. They also can be used as immunogens to produce antibodies. Thes ... antibodies can be, for example, polyclonal or monoclonal antibodies, chimeri ... ngle chain and humanized antibodies, as well as Fab fragments, or the product of ... Fab expression library. Various procedures known in the art may be used for th ... oduction of such antibodies and fragments.

For example, antibodies generated against a pol ... tide comprising a sequence of the present invention can be obtained by di ... jection of the polypeptide into an animal or by administering the poly ... tide to an animal such as a

mouse, [illegible]oat or human. A mouse, rabbit or goat is preferred. The polypep[illegible]lected from the group consisting of SEQUENCE ID NOS 14-19, and frag[illegible]hereof. The antibody so obtained then will bind the polypeptide itself. In [illegible]anner, even a sequence encoding only a fragment of the polypeptide can be u[illegible]nerate antibodies that bind the native polypeptide. Such antibodies then can [illegible]d to isolate the polypeptide from test samples such as tissue suspected of contain[illegible]hat polypeptide. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique as described by Kohler and Milstein, Nature 256:495-497 (1975), the trioma technique, the human B-cell hybridoma technique as described by Kozbor et al, Immun. Today 4:72 (1983) and the EBV-hybridoma technique to produce human monoclonal antibodies as described by Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc, New York, NY, pp. 77-96 (1985). Techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. See, for example, U.S. Patent No. 4,946,778.

Various assay formats may utilize the antibodies of the present invention, including "sandwich" immunoassays and probe assays. For example, the antibodies of the present invention, or fragments thereof, can be employed in various assay systems to determine the presence, if any, of LU103 antigen in a test sample. For example, in a first assay format, a polyclonal or monoclonal antibody or fragment thereof, or a combination of these antibodies, which has been coated on a solid phase, is contacted with a test sample, to form a first mixture. This first mixture is incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, or a combination of these antibodies, to which a signal generating compound has been attached, is contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of LU103 antigen in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the signal generating compound. The amount of LU103 antigen present in the test sample is proportional to the signal generated.

In an alternative assay format, a mixture is formed by contacting: (1) a polyclonal antibody, monoclonal antibody, or fragment thereof, which specifically binds to LU103 antigen, or a combination of such antibodies bound to a solid support; (2) the test sample; and (3) an indicator reagent comprising a monoclonal antibody, polyclonal antibody, or fragment thereof, which specifically binds to a different LU103 antigen (or a combination of these antibodies) to which a signal generating compound is attached. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence, if any, of LU103 antigen present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating compound. The amount of LU103 antigen present in the test sample is proportional to the signal generated.

In another assay format, one or a combination of at least two monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to LU103 antigen. For example, LU103 polypeptides such as the recombinant antigens disclosed herein, either alone or in combination, are coated on a solid phase. A test sample suspected of containing antibody to LU103 antigen then is incubated with an indicator reagent comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent bound to the solid phase or the indicator reagent bound to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured.

In yet another detection method, each of the monoclonal or polyclonal antibodies of the present invention can be employed in the detection of LU103 antigens in tissue sections, as well as in cells, by immunohistochemical analysis. Cytochemical analysis wherein these antibodies are labeled directly (with, for example, fluorescein, colloidal gold, horseradish peroxidase, alkaline phosphatase, etc.) or are labeled by using secondary labeled anti-species antibodies (with various labels as exemplified herein) to track the histopathology of disease also are within the scope of the present invention.

In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific LU103 polypeptides from cell cultures or biological tissues such as to purify recombinant and native LU103 proteins.

The monoclonal antibodies of the invention al used for the generation of chimeric antibodies for therapeutic use, similar applications.

The monoclonal antibodies or fragments there provided individually to detect LU103 antigens. Combinations of the mono ntibodies (and fragments thereof) provided herein also may be used as components in a mixture or "cocktail" of at least one LU103 antibody vention, along with antibodies which specifically bind to other LU103 re ach antibody having different binding specificities. Thus, this cocktail can le the monoclonal antibodies of the invention which are directed to LU103 lypeptides disclosed herein and other monoclonal antibodies specific to other ntigenic determinants of LU103 antigens or other related proteins.

The polyclonal antibody or fragment thereof whi h can be used in the assay formats should specifically bind to an LU103 polypepti or other LU103 polypeptides additionally used in the assay. The polycl l antibody used preferably is of mammalian origin such as, human, go bbit or sheep polyclonal antibody which binds LU103 polypeptide. Most prefer the polyclonal antibody is of rabbit origin. The polyclonal antibodies used in t says can be used either alone or as a cocktail of polyclonal antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different binding specificity to LU103 polypeptides, they are useful for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to, diseases and conditions of the lung such as lung cancer.

It is contemplated and within the scope of the present invention that LU103 antigen may be detectable in assays by use of a recombinant antigen as well as by use of a synthetic peptide or purified peptide, which peptide comprises an amino acid sequence of LU103. The amino acid sequence of such a polypeptide is selected from the group consisting of SEQUENCE ID NOS 14-19, and fragments thereof. It also is within the scope of the present invention that different synthetic, recombinant or purified peptides, identifying different epitopes of LU103, can be used in combination in an assay for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to diseases and conditions of the lung, such as lung cancer. In this case, all of these peptides can be coated onto one solid phase; or each separate peptide may be coated onto separate solid phases, such as microparticles, and then combined to form a mixture

of peptides w be later used in assays. Furthermore, it is contemplated that multiple pept ch define epitopes from different antigens may be used for the detection, di staging, monitoring, prognosis, prevention or treatment of, or determining t lisposition to, diseases and conditions of the lung, such as lung cancer. Pepti ed on solid phases or labeled with detectable labels are then allowed to co pete with those present in a patient sample (if any) for a limited amount of ant body. A reduction in binding of the synthetic, recombinant, or purified peptides to the antibody (or antibodies) is an indication of the presence of LU103 antigen in the patient sample. The presence of LU103 antigen indicates the presence of lung tissue disease, especially lung cancer, in the patient. Variations of assay formats are known to those of ordinary skill in the art and many are discussed herein below.

In another assay format, the presence of anti-LU103 antibody and/or LU103 antigen can be detected in a simultaneous assay, as follows. A test sample is simultaneously contacted with a capture reagent of a first analyte, wherein said capture reagent comprises a first binding member specific for a first analyte attached to a solid phase and a capture reagent for a second analyte, wherein said capture reagent comprises a first binding member for a second analyte attached to a second solid phase, to thereby form a mixture. This mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second analyte complexes. These so-formed complexes then are contacted with an indicator reagent comprising a member of a binding pair specific for the first analyte labeled with a signal generating compound and an indicator reagent comprising a member of a binding pair specific for the second analyte labeled with a signal generating compound to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte/indicator reagent complexes and capture reagent/second analyte/indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated in connection with the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample. In this assay format, recombinant antigens derived from the expression systems disclosed herein may be utilized, as well as monoclonal antibodies produced from the proteins derived from the expression systems as disclosed herein. For example, in this assay system, LU103 antigen can be the first analyte. Such assay systems are described in greater detail in EP Publication No. 0473065.

In yet other assay formats, the polypeptides disclosed herein may be utilized to detect the presence of antibody against LU103 antigen in test samples. For example, a test sample is incubated with a solid phase to which at least one polypeptide such as a recombinant protein or synthetic peptide has been attached. The polypeptide is selected from the group consisting of SEQUENCE ID NOS 14-19, and fragments thereof. These are reacted for a time and under conditions sufficient to form antigen/antibody complexes. Following incubation, the antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen. In another assay format, a test sample is contacted with a solid phase to which a recombinant protein produced as described herein is attached, and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labeled with an indicator reagent. After incubation for a time and under conditions sufficient for antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free phase as an indication of the presence of antibody against LU103 antigen. Other assay formats utilizing the recombinant antigens disclosed herein are contemplated. These include contacting a test sample with a solid phase to which at least one antigen from a first source has been attached, incubating the solid phase and test sample for a time and under conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labeled antigen, which antigen is derived from a second source different from the first source. For example, a recombinant protein derived from a first source such as E. coli is used as a capture antigen on a solid phase, a test sample is added to the so-prepared solid phase, and following standard incubation and washing steps as deemed or required, a recombinant protein derived from a different source (i.e., non-E. coli) is utilized as a part of an indicator reagent which subsequently is detected. Likewise, combinations of a recombinant antigen on a solid phase and synthetic peptide in the indicator phase also are possible. Any assay format which utilizes an antigen specific for LU103 produced or derived from a first source as the capture antigen and an antigen specific for LU103 from a different second source is contemplated. Thus, various combinations of recombinant antigens, as well as the use of synthetic peptides, purified proteins and the like, are within the scope of this invention. Assays such as this and others are described in U.S. Patent No. 5,254,458, which enjoys common ownership herewith.

Other embodiments which utilize various othe phases also are contemplated and are within the scope of this inventic example, ion capture procedures for immobilizing an immobilizable reactio: plex with a negatively charged polymer (described in EP publication 03261C EP publication No. 0406473), can be employed according to the present i n to effect a fast solution-phase immunochemical reaction. An immobi e immune complex is separated from the rest of the reaction mixture by ionic actions between the negatively charged poly-anion/immune complex and t viously treated, positively charged porous matrix and detected by usin ous signal generating systems previously described, including those describe chemiluminescent signal measurements as described in EPO Publication No. 0 .115.

Also, the methods of the present invention can dapted for use in systems which utilize microparticle technology including autom and semi-automated systems wherein the solid phase comprises a micropar (magnetic or non-magnetic). Such systems include those described in, ample, published EPO applications Nos. EP 0 425 633 and EP 0 424 634, re ively.

The use of scanning probe microscopy (SPM) immunoassays also is a technology to which the monoclonal antibodies of the p sent invention are easily adaptable. In scanning probe microscopy, particularly in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunneling microscopy elim nates the need for labels which normally must be utilized in many immunoassa tems to detect antigen/antibody complexes. The use of SPM to moni pecific binding reactions can occur in many ways. In one embodiment, one me r of a specific binding partner (analyte specific substance which is the monoc l antibody of the invention) is attached to a surface suitable for scanning The attachment of the analyte specific substance may be by adsorption to a t ece which comprises a solid phase of a plastic or metal surface, following me known to those of ordinary skill in the art. Or, covalent attachment of a s fic binding partner (analyte specific substance) to a test piece which test p comprises a solid phase of derivatized plastic, metal, silicon, or glass may be util Covalent attachment methods are known to those skilled in the art and incl variety of means to irreversibly link specific binding partners to the test pi . If the test piece is silicon

c[illegible] the surface must be activated prior to attaching the specific binding partner. A[illegible]olyelectrolyte interactions may be used to immobilize a specific binding p[illegible]er on a surface of a test piece by using techniques and chemistries. The p[illegible]ferred method of attachment is by covalent means. Following attachment of a s[illegible]ific binding member, the surface may be further treated with materials such as s[illegible]um. proteins, or other blocking agents to minimize non-specific binding. The s[illegible]face also may be scanned either at the site of manufacture or point of use to verify it[illegible] suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

While the present invention discloses the preference for the use of solid phases, it is contemplated that the reagents such as antibodies, proteins and peptides of the present invention can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the present invention.

It is contemplated that the reagent employed for the assay can be provided in the form of a test kit with one or more containers such as vials or bottles, with each c[illegible]ntainer containing a separate reagent such as a probe, primer, monoclonal antibody or a cocktail of monoclonal antibodies, or a polypeptide (e.g. recombinantly, synthetically produced or purified) employed in the assay. The polypeptide is selected from the group consisting of SEQUENCE ID NOS 14-19, and fragments thereof. Other components such as buffers, controls and the like, known to those of ordinary skill in art, may be included in such test kits. It also is contemplated to provide test kits which have means for collecting test samples comprising accessible body fluids, e.g., blood, urine, saliva and stool. Such tools useful for collection ("collection materials") include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or c[illegible]ntain preservatives, stabilizers or antimicrobial agents to help maintain the integrity c[illegible] the specimens. Test kits designed for the collection, stabilization and preservation c[illegible] test specimens obtained by surgery or needle biopsy are also useful. It is c[illegible]ntemplated that all kits may be configured in two components which can be p[illegible]ovided separately; one component for collection and transport of the specimen and the other component for the analysis of the specimen. The collection component, for

example, can be provided to the open market user while the components for analy can be provided to others such as laboratory personnel for determination of the presence, absence or amount of analyte. Further, kits for the collection, stabiliza and preservation of test specimens may be configured for use by untrained perso and may be available in the open market for use at home with subsequent transportation to a laboratory for analysis of the test sample.

E. coli bacteria (clone 1235095) has been deposited at the American Type Culture Collection (A.T.C.C.), 12301 Parklawn Drive, Rockville, Maryland 208[illegible]2, as of 10/7/96, under the terms of the Budapest Treaty and will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, or for the enforceable period of the U.S. patent, whichever is longer. The deposit and any other deposited material described herein are provided for convenience only, and are not required to practice the present invention in view of the teachings provided herein. The cDNA sequence in all of the deposited material is incorporated herein by reference. Clone 1235095 was accorded A.T.C.C. Deposit No. 98184.

The present invention will now be described by way of examples, which are meant to illustrate, but not to limit, the scope of the present invention.

EXAMPLES

Example 1: Identification of Lung Tissue Library LU103 Gene-Specific Clones

A. Library Comparison of Expressed Sequence Tags (ESTs) or Transcript Images. Partial sequences of cDNA clone inserts, so-called "expressed sequence tags" (ESTs), were derived from cDNA libraries made from lung tumor tissues, lung non-tumor tissues and numerous other tissues, both tumor and non-tumor and entered into a database (LIFESEQ™ database, available from Incyte Pharmaceuticals, Palo Alto, CA) as gene transcript images. See International Publication No. WO 95/20681. (A transcript image is a listing of the number of ESTs for each of the represented genes in a given tissue library. ESTs sharing regions of mutual sequence overlap are classified into clusters. A cluster is assigned a clone number from a representative 5' EST. Often, a cluster of interest can be extended by comparing its consensus sequence with sequences of other ESTs which did not meet the criteria for automated clustering. The alignment of all available clusters and single ESTs represent a contig from which a consensus sequence is derived.) The transcript images then were evaluated to identify EST clusters that

were representative primarily of the lung tiss ies. These target clusters then were ranked according to their abundance (oc e of EST members) in the target libraries and their absence from background l . Higher abundance clusters with low background occurrence were given study priority. Overlapping clones 1235095 (SEQUENCE ID NO 1), 12: SEQUENCE ID NO 2), and 1379417 (SEQUENCE ID NO 3) were ident r further study. These represented the minimum number of clones th needed to form the LU103 contig and from which the consensus sequen vided herein (SEQUENCE ID NO 4) was derived.

B. Generation of a Consensus Seque: The nucleotide sequences of EST clones, 1235095 (SEQUENCE ID NO 1), 12. 1 (SEQUENCE ID NO 2), and 1379417 (SEQUENCE ID NO 3), were enter the Sequencher™ Program (available from Gene Codes Corporation, An bor, MI, in order to generate a nucleotide alignment (contig map) and then g te their consensus sequence (SEQUENCE ID NO 4). FIGURE 1 shows t cleotide sequence alignment of these clones and their resultant nucleotide co sequence (SEQUENCE ID NO 4). FIGURE 2 presents the contig map depic clones SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO rming overlapping regions of the LU103 gene and the resultant consensus nucle de sequence (SEQUENCE ID NO 4) of these clones in a graphic display. Following this, a three-frame translation was performed on the consensus sequence (SEQUENCE ID NO 4). The third forward frame was found to have an open reading frame encoding a 93 residue amino acid sequence, which is presented as SEQUENCE ID NO 14.

C. Specificity of Expression of ESTs esponding to Consensus Sequence. The consensus sequence, generate section B, supra, was compared to the entire updated LIFESEQ™ database (Se mber 1997) using the BLAST search tool. ESTs corresponding to the conse sequence were found in 66.6% (22 of 33) of lung libraries and 0.2% (1 of 45 of non-lung libraries. Therefore, the consensus sequence or fragment thereof v und more than 302 times more often in lung than non-lung tissues.

Example 2: Sequencing of 3 EST-Specific Clones

The DNA sequence of clone 1235095 h comprises the 5'-most EST of the LU103 gene contig was determined using y termination sequencing with

erminators following known methods (SEQUENCE ID NO 5). (F. Sanger et NAS U.S.A. 74:5463 (1977)).

Because the pINCY vector (Life Technologies, Gaithersburg, MD) contains ersal priming sites just adjacent to the 3' and 5' ligation junctions of the inserts, ximately 300 bases of the insert were sequenced in both directions using the versal primers depicted in SEQUENCE ID NO 8 and SEQUENCE ID NO 9 ained from New England Biolabs, Beverly, MA and Applied Biosystems Inc, ter City, CA, respectively). The sequencing reactions were run on a lyacrylamide denaturing gel, and the sequences were determined by an Applied Biosystems 377 Sequencer (available from Applied Biosystems, Foster City, CA) or other sequencing apparatus. Additional sequencing primers, SEQUENCE ID NO 10 and SEQUENCE ID NO 11, were designed from sequence information determined by the initial sequencing reactions near the 3'-ends of the two DNA strands. These primers then were used to determine the remaining DNA sequence of the cloned insert from each DNA strand, as previously described.

Example 3: Nucleic Acid Preparation

A. RNA Extraction from Tissue. Total RNA was isolated from solid lung tissues or cells and from non-lung tissues. Various methods were utilized, including but not limited to the lithium chloride/urea technique, known and described in the art (Kato et al., J. Virol. 61:2182-2191, (1987)), Ultraspec™ (Biotecx Laboratories, Inc., Houston Texas), and TRIzol™ (Life Technologies, Inc., Gaithersburg, MD).

For northern blot analysis, the tissue was placed in a sterile conical tube on ice and 10-15 volumes of 3 M LiCl, 6 M urea, 5 mM EDTA, 0.1 M β-mercaptoethanol, 50 mM Tris-HCl (pH 7.5) were added. The tissue was homogenized with a Polytron® homogenizer (Brinkman Instruments, Inc., Westbury, NY) for 30-50 seconds on ice. The solution was transferred to a 15 ml plastic centrifuge tube and placed overnight at -20°C. The tube was centrifuged for min at 9,000 x g at 0-4°C, and the supernatant was immediately decanted. Then, ml of 3 M LiCl were added, the tube was vortexed for 5 sec and centrifuged for min at 11,000 x g at 0-4°C, and the above decanting, resuspension in LiCl, and centrifugation steps were repeated. The final pellet was air-dried and resuspended in ml of 1 mM EDTA, 0.5% SDS, 10 mM Tris (pH 7.5). Next, a 20 µl volume of proteinase K (20 mg/ml) was added, and the solution incubated for 30 min at 37°C with occasional mixing. One-tenth volume (0.22-0.25 ml) of 3 M NaCl was added

and the solution was vortexed before transfer into another tube which contained ul of phenol/chloroform/isoamyl alcohol (PCI). The tube was vortexed for 1-3 se d centrifuged for 20 min at 3,000 x g at 10°C, after which the PCI extraction was repeated twice, then followed by two similar extractions with chloroform/isoam alcohol. The final aqueous solution was transferred to a pre-chilled 15 ml corex glass tube containing 6 ml of 100% absolute ethanol, the tube was covered with parafilm and placed at -20°C overnight. The tube was centrifuged for 30 min at 10,000 x g at 0-4°C, and the ethanol supernatant was decanted immediately. Th RNA pellet was washed four times with 10 ml of 75% ice-cold ethanol, followe each time by centrifugation at 10,000 x g for 10 min. The final pellet was air-di ed for 15 min at room temperature. The RNA was suspended in 0.5 ml of 10 mM Tris (pH 7.6), 1 mM EDTA, and its concentration was determined spectrophotometrically. RNA samples were aliquoted and stored at -70°C as eth anol precipitates.

The quality of the RNA was determined by agarose gel electrophoresis (see Example 5) and staining with 0.5 µg/ml ethidium bromide for one hour. RNA samples that did not contain intact 28S/18S rRNAs were excluded from the stud y.

Alternatively, for RT-PCR analysis, 1 ml of Ultraspec RNA reagent was added to 120 mg of pulverized tissue in a 2.0 ml polypropylene microfuge tube, homogenized with a Polytron® homogenizer (Brinkman Instruments, Inc., Westbury, NY) for 50 sec and left on ice for 5 min. 0.2 ml of chloroform was then added to each sample, followed by vortexing for 15 sec. The sample was left in ice for another 5 min, followed by centrifugation at 12,000 x g for 15 min at 4°C. The upper layer was collected and transferred to another RNase-free 2.0 ml microfuge tube. An equal volume of isopropanol was added to each sample, and the solution was placed on ice for 10 min. The sample was centrifuged at 12,000 x g for 10 min at 4°C, and the supernatant was discarded. The remaining pellet was washed twice with cold 75% ethanol, resuspended by vortexing, and the resuspended material was then re-pelleted by centrifugation at 7500 x g for 5 min at 4°C. Finally, the RNA pellet was dried in a speedvac for at least 5 min and reconstituted in RNase-free water.

B. RNA Extraction from Blood Mononuclear Cells. Mononuclear cells are isolated from blood samples from patients by centrifugation using Ficoll-Hypaque as follows. A 10 ml volume of whole blood is mixed with an equal volume of RPMI Medium (Life Technologies, Gaithersburg, MD). This mixture is then underlayed

with 10 ml of Ficoll-Hypaque (Pharmac ıtaway, NJ) and centrifuged for 30 minutes at 200 x g. The buffy coat cont: he mononuclear cells is removed, diluted to 50 ml with Dulbecco's PBS (L hnologies, Gaithersburg, MD) and the mixture centrifuged for 10 minutes a g. After two washes, the resulting pellet is resuspended in Dulbecco's PBS al volume of 1 ml.

RNA is prepared from the isolate onuclear cells as described by N. Kato et al., supra. Briefly, the pelleted r uclear cells are brought to a final of 1 ml volume and then are resuspended in 2 L of PBS and mixed with 2.5 ml of 3M LiCl, 6M urea, 5mM EDTA, 0.1M 2 aptoethanol, 50mM Tris-HCl (pH 7.5). The resulting mixture is homogeni: id incubated at -20°C overnight. The homogenate is spun at 8,000 RPM in a B an J2-21M rotor for 90 minutes at 0-4°C. The pellet is resuspended in 10 ml LiCl by vortexing and then spun at 10,000 RPM in a Beckman J2-21M roto rifuge for 45 minutes at 0-4°C. The resuspending and pelleting steps then are ated. The pellet is resuspended in 2 ml of 1 mM EDTA, 0.5% SDS, 10 mM Tri 7.5) and 400 µg Proteinase K with vortexing and then it is incubated at 37°C 30) minutes with shaking. One tenth volume of 3M NaCl then is added and th exed mixture. Proteins are removed by two cycles of extraction with phenol/ roform/ isoamyl alcohol followed by one extraction with chloroform/ isoamyl hol. RNA is precipitated by the addition of 6 ml of ethanol followed by o night incubation at -20°C. After the precipitated RNA is collected by centrifu ation, the pellet is washed 4 times in 75% ethanol. The pelleted RNA is then dissol ed in 1mM EDTA, 10mM Tris-HCl (pH 7.5).

Non-lung tissues are used as neg controls. The mRNA can be further purified from total RNA by using comm lly available kits such as oligo dT cellulose spin columns (RediCol™ from rmacia, Uppsala, Sweden) for the isolation of poly-adenylated RNA. Tota mRNA can be dissolved in lysis buffer (5M guanidine thiocyanate, 0.1M EDTA H 7.0) for analysis in the ribonuclease protection assay.

C. RNA Extraction from polyso . Tissue is minced in saline at 4°C and mixed with 2.5 volumes of 0.8 M sucro n $TK_{150}M$ (150 mM KCl, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.4) soluti ntaining 6 mM 2-mercaptoethanol. The tissue is homogenized in a Teflon-g Potter homogenizer with five strokes at 100-200 rpm followed by six strokes in nce homogenizer, as described by B. Mechler, Methods in Enzymology 152:2 48 (1987). The homogenate then is

rifuged at 12,000 x g for 15 min at 4°C to sediment the nuclei. The polysomes solated by mixing 2 ml of the supernatant with 6 ml of 2.5 M sucrose in $TK_{150}M$ layering this mixture over 4 ml of 2.5 M sucrose in $TK_{150}M$ in a 38 ml vallomer tube. Two additional sucrose $TK_{150}M$ solutions are successively ed onto the extract fraction; a first layer of 13 ml 2.05 M sucrose followed by a ond layer of 6 ml of 1.3 M sucrose. The polysomes are isolated by centrifuging gradient at 90,000 x g for 5 h at 4°C. The fraction then is taken from the 1.3 M rose/2.05 M sucrose interface with a siliconized pasteur pipette and diluted in an qual volume of TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). An equal volume of 90°C SDS buffer (1% SDS, 200 mM NaCl, 20 mM Tris-HCl, pH 7.4) is added and the solution is incubated in a boiling water bath for 2 min. Proteins next are digested with a Proteinase-K digestion (50 mg/ml) for 15 min at 37°C. The mRNA is purified with 3 equal volumes of phenol-chloroform extractions followed by precipitation with 0.1 volume of 2 M sodium acetate (pH 5.2) and 2 volumes of 100% ethanol at -20°C overnight. The precipitated RNA is recovered by centrifugation at 12,000 x g for 10 min at 4°C. The RNA is dried and resuspended in TE (pH 7.4) or distilled water. The resuspended RNA then can be used in a slot blot or dot blot hybridization assay to check for the presence of LU103 mRNA (see Example 6).

The quality of nucleic acid and proteins is dependent on the method of preparation used. Each sample may require a different preparation technique to maximize isolation efficiency of the target molecule. These preparation techniques are within the skill of the ordinary artisan.

Example 4: Ribonuclease Protection Assay

A. Synthesis of Labeled Complementary RNA (cRNA) Hybridization Probe and Unlabeled Sense Strand. A plNCY plasmid containing the LU103 gene cDNA sequence insert (clone 1235095), flanked by opposed SP6 and T7 polymerase promoters, was purified using Qiagen Plasmid Purification Kit (Qiagen, Chatsworth, CA). 10 µg of the resulting plasmid were then cut with 10 U DdeI restriction enzyme for 1 h at 37°C. The cut plasmid was purified using QIAprep kits (Qiagen, Chatsworth, CA) and used for the synthesis of antisense transcript which is labeled with 6.3 µM (alpha^{32}P) UTP (800 Ci/mmol)(Amersham Life Sciences, Inc. Arlington Heights, IL) from the SP6 promoter using the Riboprobe® in vitro Transcription System (Promega Corporation, Madison, WI) according to the

supplier's instructions. To generate the sense strand, 10 µg of the purified pla... were cut with restriction enzymes 10U XbaI and 10 U NotI, and transcribed a... above from the T7 promoter. Both sense and antisense strands were isolated b... ...n column chromatography. Unlabeled sense strand was quantitated by UV absor... ...a at 260 nm.

B. Hybridization of Labeled Probe to Target. Frozen tissue was pulveri... ...d to powder under liquid nitrogen and 100-500 mg were dissolved in 1 ml of lysis buffer as available as a component of the DirectProtect™ Lysate RNase Protectio... kit (Ambion, Inc., Austin, TX). Further dissolution was achieved using a tissue homogenizer. In addition, a dilution series of a known amount of sense strand i... mouse liver lysate was made for use as a positive control. Finally, 45 µl of solubilized tissue or diluted sense strand was mixed directly with 1 x 10^5 cpm of radioactively labeled probe (1.5 x 10^9 cpm/µg) in 5 µl of lysis buffer. Hybridiza...ion was allowed to proceed overnight at 37°C.

C. RNase Digestion. RNA that was not hybridized to probe was remov... from the reaction as per the Direct Protect™ protocol using a solution of RNase A and RNase T1 for 30 min at 37°C, followed by removal of RNase by Proteinase-digestion in the presence of sodium sarcosyl. Hybridized fragments protected fr...m digestion were then precipitated by the addition of an equal volume of isopropanol and placed at -70°C for 3 h. The precipitates were collected by centrifugation at 12,000 x g for 20 min.

D. Fragment Analysis. The precipitates were dissolved in denaturing gel loading dye (80% formamide, 10 mM EDTA (pH 8.0), 1 mg/ml xylene cyanol, ... mg/ml bromophenol blue), heat denatured, and electrophoresed in 6% polyacrylamide TBE, 8 M urea denaturing gels. The gels were imaged and analy...ed using the STORM™ storage phosphor autoradiography system (Molecular Dynamics, Sunnyvale, CA). Quantitation of protected fragment bands, expresse... in femtograms (fg), was achieved by comparing the peak areas obtained from the test samples to those from the known dilutions of the positive control sense strand (s...e Section B, supra). In addition, the concentration of DNA in the lysate was assay...d to estimate the number of cells in the test sample lysates. The results are expres...d in molecules of LU103 RNA/cell and as a image rating score (Table 1). High le...i expression of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID N... 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements

thereof, indicated the presence of LU1 .A(s), suggesting a diagnosis of a lung tissue disease or condition, such a ncer.

Tissue	ID Number		RNA/cell	Score*
Normal Lung	C157		10	+
	C007G		7	+
	C027R		8	+
	C016R		2	+
	C135R		0.2	+
Malignant Lung	C011G		>46	3+
	C023G		0.3	+
	C012G		0	-
	C033R		>87	3+
	C030		0	-
Normal Lung	C005R		0	-
Malignant Lung	C037G		0	-
Normal Colon	C027G		0	-

*Samples with no detectable protected fr... were scored as "-"; samples with detectable protected fragment (the fg valu... which were within the std curve) were scored as "+"; samples with detectable pr... fragment and having fg values 2 to 10 fold above the std curve were scored as ...+"; and samples with detectable protected fragment having fg values 10 fol... more above the std curve were scored as "3+".

Example 5: Northern Blotting

The northern blot technique was used to identify a specific size RNA species in a complex population of RNA using agar... gel electrophoresis and nucleic acid hybridization. Briefly, 5-10 µg of total RN... (see Example 3) was incubated in 15 µl of a solution containing 40 mM morphil...propanesulfonic acid (MOPS) (pH 7.0), 10 mM sodium acetate, 1 mM EDTA, ...2 M formaldehyde, 50% v/v formamide for 15 min at 65°C. The denatu... RNA was mixed with 2 µl of loading buffer (50% glycerol, 1 mM EDTA, 0.4% ...romophenol blue, 0.4% xylene cyanol) and loaded into a denaturing 1.0% agaros... containing 40 mM MOPS (pH 7.0), 10 mM sodium acetate, 1 mM EDTA and ... M formaldehyde. The gel was electrophoresed at 60 V for 1.5 h and rins... in RNAse free water. Gels were stained with 0.5 µg/ml of ethidium brom... RNAse free water and illuminated with UV light to visualize ribosomal RNA ...nds. RNA was transferred from the gel onto nylon membranes (Brightstar-Plu... ...ion, Inc., Austin, TX) for 1.5 hours using the downward alkaline capillary tran... method (Chomczynski, Anal.

Bi :201:134-139, 1992). The filter was rinsed with 1X SSC, and RNA was cr to the filter using a Stratalinker (Stratagene, Inc., La Jolla, CA) on the aut linking mode and dried for 15 min. The membrane was then placed into a hy n tube containing 20 ml of preheated prehybridization solution (5X SSC, 50 mide, 5X Denhardt's solution, 100 µg/ml denatured salmon sperm DNA) anc ated in a 42°C hybridization oven for at least 3 hr. While the blot was pre ing, a ^{32}P-labeled random-primed probe was generated using the LU103 inse gment (obtained by digesting clone 1235095 with XbaI and NotI) using Ran Primer DNA Labeling System (Life Technologies, Inc., Gaithersburg, MD) acc g to the manufacturer's instructions. Half of the probe was boiled for 10 min, quick chilled on ice and added to the hybridization tube. Hybridization was carr out at 42°C for at least 12 hr. The hybridization solution was discarded and the filter was washed in 30 ml of 3X SSC, 0.1% SDS at 42°C for 15 min, followed by 30 ml of 3X SSC, 0.1% SDS at 42°C for 15 min. The filter was wrapped in sar p, exposed to Kodak XAR-Omat film for 8-96 hr, and the film was dev d for analysis.

Results of the analysis of RNA quality using an ethidium bromide stained agarose gel and the corresponding northern blot using LU103 probe hybridized to RNAs from non-lung tissues and lung tissues are shown in Figure 3A and Figure 3B, respectively. The positions of RNA size standards (in kb) are shown to the left of each panel. As shown in Figure 3A, the LU103 probe detected a 0.75 kb band in RNA isolated from lung tissue (lane 6) but did not detect a band in the RNA isolated from 11 other tissues. Figure 3B shows that the LU103 probe detected a 0.75 kb ban the RNA of 5/6 normal lung tissues and 4/6 lung cancer tissues. The 0.75 kb b d was not detected in the lung sample of lane 3, nor in the seven non-lung sam s in lanes 4-10 (which contained colon, colon, lung, lung, ovary, prostate, and spleen tissue, respectively). Lane 2 was blank.

Example 6: Dot Blot/Slot Blot

Dot and slot blot assays are quick methods to evaluate the presence of a spe nucleic acid sequence in a complex mix of nucleic acid. To perform such ass p to 50 µg of RNA is mixed in 50 µl of 50% formamide, 7% for hyde, 1X SSC, incubated 15 min at 68°C, and then cooled on ice. Then, 10t 20X SSC is added to the RNA mixture and loaded under vacuum onto a man old apparatus that has a prepared nitrocellulose or nylon membrane. The

membrane is soaked in water, 20X SSC for 1 hour, placed on two sheets of 20X SSC prewet Whatman #3 filter paper, and loaded into a slot blot or dot blot vacuum manifold apparatus. The slot blot is analyzed with probes prepared and labeled as described in Example 4, supra. Detection of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof, is an indication of the presence of LU103, suggesting a diagnosis of a lung tissue disease or condition, such as lung cancer.

Other methods and buffers which can be utilized in the methods described in Examples 5 and 6, but not specifically detailed herein, are known in the art and are described in J. Sambrook et al, supra.

Example 7: In Situ Hybridization

This method is useful to directly detect specific target nucleic acid sequences in cells using detectable nucleic acid hybridization probes.

Tissues are prepared with cross-linking fixative agents such as paraformaldehyde or glutaraldehyde for maximum cellular RNA retention. See, L. Angerer et al., Methods in Cell Biol. 35:37-71 (1991). Briefly, the tissue is placed in greater than 5 volumes of 1% glutaraldehyde in 50 mM sodium phosphate, pH 7.5 at 4°C for 30 min. The solution is changed with fresh glutaraldehyde solution (1% glutaraldehyde in 50mM sodium phosphate, pH 7.5) for a further 30 min fixing. The fixing solution should have an osmolality of approximately 0.375% NaCl. The tissue is washed once in isotonic NaCl to remove the phosphate.

The fixed tissues then are embedded in paraffin as follows. The tissue is dehydrated though a series of ethanol concentrations for 15 min each: 50% (twice), 70% (twice), 85%, 90% and then 100% (twice). Next, the tissue is soaked in two changes of xylene for 20 min each at room temperature. The tissue is then soaked in two changes of a 1:1 mixture of xylene and paraffin for 20 min each at 60°C; and then in three final changes of paraffin for 15 min each.

Next, the tissue is cut in 5 µm sections using a standard microtome and placed on a slide previously treated with a tissue adhesive such as 3-aminopropyltriethoxysilane.

Paraffin is removed from the tissue by two 10 min xylene soaks and rehydrated in a series of ethanol concentrations: 99% twice, 95%, 85%, 70%, 50%,

30%, and then distilled water twice. Th... ...ns are pre-treated with 0.2 M HCl for 10 min and permeabilized with 2 µg/ml ... ...sc-K at 37°C for 15 min.

Labeled riboprobes transcribed f... ...LU103 gene plasmid (see Example 4) are hybridized to the prepared tissue s... ...and incubated overnight at 56°C in 3X standard saline extract and 50% form... Excess probe is removed by washing in 2X standard saline citrate and... ...formamide followed by digestion with 100 µg/ml RNase A at 37°C for 30... Probe fluorescence is visualized by illumination with ultraviolet (UV) light u... ...microscope. Fluorescence in the cytoplasm is indicative of LU103 mRNA... ...ternatively, the sections can be visualized by autoradiography.

Example 8: Reverse Transcription PCR

A. One Step RT-PCR Assay. Tar... ...specific primers are designed to detect the above-described target sequences by ... ...se transcription PCR using methods known in the art. One step RT-PCR is a ... ...ential procedure that performs both RT and PCR in a single reaction mixture. Th... ...cedure is performed in a 200 µl reaction mixture containing 50 mM (N,N-... ...2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM ethylene diaminetetraacetic acid, 0.02 mg/ml NaN_3, 8% w/v glycerol, 150 µM each of dNTP, 0.25 µM each primer, 5U rTth polymerase, 3.25 mM $Mn(OAc)_2$ and 5 µl of target RNA (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of $Mn(OAc)_2$, the $Mn(OAc)_2$ should be added just before target addition. Optimal conditions for cDNA synthesis and thermal cycling readily can be determined by those skilled in the ar... The reaction is incubated in a Perkin-Elmer Thermal Cycler 480. Optimal con...ns for cDNA synthesis and thermal cycling can readily be determined by tho... ...illed in the art. Conditions which may be found useful include cDNA synthesis a... ...°-70°C for 15-45 min and 30-45 amplification cycles at 94°C, 1 min; 55°-70°C, 1 min; 72°C, 2 min. One step RT-PCR also may be performed by using a d... ...enzyme procedure with Taq polymerase and a reverse transcriptase enzyme, such ... ...MMLV or AMV RT enzymes.

B. Traditional RT-PCR. A tradi... ...l two-step RT-PCR reaction was performed using the method of K.Q. Hu ... ...l., Virology 181:721-726 (1991). Briefly, 0.5 µg of extracted mRNA (see ... ...ple 3) was reverse transcribed in a 20 µl reaction mixture containing 1X PCR ... ...er (Perkin-Elmer), 5 mM $MgCl_2$, 1 mM dNTP, 20 U RNasin, 2.5 µM random ... ...examers, and 50 U MMLV (Moloney

mu ukemia virus) reverse transcriptase (RT). Reverse transcription was pe l at room temperature for 10 min, 42°C for 60 min in a PE-480 thermal cy llowed by further incubation at 95°C for 5 min to inactivate the RT. PCR wa ormed using 2 µl of the cDNA reaction in a final PCR reaction volume of 50 µl aing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM dN 0.5 µM of each sense and antisense primer (SEQUENCE ID NO 12 and SE NCE ID NO 13, respectively), and 2.5 U of Taq polymerase. The reaction wa ubated in an MJ Research Model PTC-200 as follows: 40 cycles of am ification (94°C, 20 sec; 58°C, 30 sec; 72°C, 30 sec); a final extension (72°C, 10 mi and a soak at 4°C.

C. PCR Fragment Analysis. The correct products were verified by size determination using gel electrophoresis. After the gel was stained with ethidium bromide (0.5 µg/ml in TBE buffer) for 15 minutes and destained in water for 10 minutes, it was visualized by UV illumination. Figure 4A, lanes 1-5, shows a DNA bar which is indicative of a β2 microglobulin-specific PCR product in both normal lun sue (lanes 1, 2, and 4) and cancerous lung tissue (lanes 3 and 5). Lanes 8-12. h contained RNA from the same specimens, shows a 269 base pair LU103-specific PCR amplification product in both normal lung tissue (lanes 8, 9, and 11) and cancerous lung tissue (lanes 10 and 12). Lane 7 shows that LU103-specific amplification of DNA with the same primers gives rise to an approximately 650 base pair fragment; thus, some of the lung RNA specimens contained DNA. Figure 4B shows that, in contrast to the LU103 RNA-specific amplification product obtained from lung tissue RNAs (lanes 1-2), the same conditions of amplification did not produce this RNA-specific product from RNAs obtained from prostate tissue (lanes 5-9), breast tissue (lanes 10-14), or colon tissue (lanes 15-19). However, many of these RNAs did contain sufficient DNA to produce a DNA-specific product. Lane 4 shows the DNA-specific amplification product control. Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof, indicated the presence of LU103 mRNA(s), suggesting a diagnosis of a lung tissue disease or condition, such as lung cancer.

Example 9: OH-PCR

A. Probe selection and Labeling. Target-specific primers and probes are designed to detect the above-described target sequences by oligonucleotide hybridization PCR. International Publication Nos WO 92/10505, published 25 June 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling oligonucleotides at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. For example, see N. T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J. S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' end to prevent participation in PCR and the formation of undesired extension products. For one step OH-PCR, the probe should have a T_M at least 15°C below the T_M of the primers. The primers and probes are utilized as specific binding members, with or without detectable labels, using standard phosphoramidite chemistry and/or post-synthetic labeling methods which are well-known to one skilled in the art.

B. One Step Oligo Hybridization PCR. OH-PCR is performed on a 200 µl reaction containing 50 mM (N,N,-bis[2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM ethylene diaminetetraacetic acid, 0.02 mg/ml NaN_3, 8% w/v glycerol, 150 µM each of dNTP, 0.25 µM each primer, 3.75 nM probe, 5U rTth polymerase, 3.25 mM $Mn(OAc)_2$ and 5 µl blood equivalents of target (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of $Mn(OAc)_2$, the $Mn(OAc)_2$ should be added just before target addition. The reaction is incubated in a Perkin-Elmer Thermal Cycler 480. Optimal conditions for cDNA synthesis and thermal cycling can be readily determined by those skilled in the art. Conditions which may be found useful include cDNA synthesis (60°C, 30 min), 30-45 amplification cycles (94°C, 40 sec; 55-70°C, 60 sec), oligo-hybridization (97°C, 5 min; 15°C, 5 min; 15°C soak). The correct reaction product contains at least one of the strands of the PCR product and an internally hybridized probe.

C. OH-PCR Product Analysis. Amplified reaction products are detected on an LCx® analyzer system (available from Abbott Laboratories, Abbott Park, IL). Briefly, the correct reaction product is captured by an antibody labeled microparticle at a capturable site on either the PCR product strand or the hybridization probe, and the complex is detected by binding of a detectable antibody conjugate to either a

detectable site on the probe or the PCR strand. Only ex containing a PCR strand hybridized with the internal probe is detectab! etection of this complex then is indicative of the presence of LU103 mRNA, ing a diagnosis of a lung disease or condition, such as lung cancer.

Many other detection formats exist which c and/or modified by those skilled in the art to detect the presence of ampli- non-amplified LU103-derived nucleic acid sequences including, but not lim ligase chain reaction (LCR, Abbott Laboratories, Abbott Park, IL); Q-beta ase (Gene-Trak™, Naperville, Illinois), branched chain reaction (Chiron ryville, CA) and strand displacement assays (Becton Dickinson, Research Tri le Park, NC).

The LU103 polynucleotides which are provide and discussed hereinabove are useful as markers of lung tissue disease, especially lung cancer. Tests based upon the appearance of this marker in a test sample su s tissue, blood, plasma or serum, can provide low cost, non-invasive, diagnostic formation to aid the physician to make a diagnosis of cancer, to help selec erapy protocol, or to monitor the success of a chosen therapy. This marke be elevated in a disease state, altered in a disease state, or be a normal protein the lung which appears in an inappropriate body compartment.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: ABBOTT LABORATORIES

TITLE OF THE INVENTION: REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE LUNG

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Abbott Laboratories
(B) STREET: 100 Abbott Park Road
(C) CITY: Abbott Park
(D) STATE: IL
(E) COUNTRY: USA
(F) ZIP: 60064-3500

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Becker, Cheryl L.
(B) REGISTRATION NUMBER: 35,441
(C) REFERENCE/DOCKET NUMBER: 5997.PC.01

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 847/935-1729
(B) TELEFAX: 847/938-2623
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 269 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCGTGG AACCACTGGC TTGGTGGATT TTGCTAGATT TTTCTGATTT TTAAACTCCT 60
GAAAAATATC CCAGATAACT GTCATGAAGC TGGTAACTAT CTTCCTGCTG GTGACCATCA 120
GCCTTTTGTAG TTACTCTGCT ACTGCCTTCC TCATCAACAA AGTGCCCCTT CCTGTTGACA 180

AGTTGGCACC TTTACCTCTG GACAACATTC TTCCCTTTAT GGATCCATTA AAGCTTCTTC 240
TGAAAACTCT GGGCATTTCT GTTGAGCAC 269

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 263 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCTACTGCC TTCCTCATCA ACAAAGTGCC CCTTCCTGTT GACAAGTTGG CACCTTTACC 60
TCTGGACAAC ATTCTTCCCT TTATGGATCC ATTAAAGCTT CTTCTGAAAA CTCTGGGCAT 120
TTCTGTTGAG CACCTTGTGG AGGGGCTAAG GAAGTGTGTA AATGAGCTGG GACCAGAGGC 180
TTCTGAAGCT GTGAAGAAAC TGCTGGAGGC GCTATCACAC TTGGTGTGAC ATCAAGATAA 240
AGAGCGGAGG TGGATGGGGA TGG 263

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 225 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGGAAGTGT GTAAATGAGC TGGGACCAGA GGCTTCTGAA GCTGTGAAGA AACTGCTGGA 60
GGCGCTATCA CACTTGGTGT GACATCAAGA TAAAGAGCGG AGGTGGATGG GGATGGAAGA 120
TGATGCTCCT ATCCTCCCTG CCTGAAACCT GTTCTACCAA TTATAGATCA AATGCCCTAA 180
AATGTAGTGA CCCGTGAAAA GGACAAATAA AGCAATGAAT ACATT 225

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 507 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGCAAGTGGA ACCACTGGCT TGGTGGATTT TGCTAGATTT TTCTGATTTT TAAACTCCTG 60
AAAAATATCC CAGATAACTG TCATGAAGCT GGTAACTATC TTCCTGCTGG TGACCATCAG 120
CCTTTGTAGT TACTCTGCTA CTGCCTTCCT CATCAACAAA GTGCCCCTTC CTGTTGACAA 180
GTTGGCACCT TTACCTCTGG ACAACATTCT TCCCTTTATG GATCCATTAA AGCTTCTTCT 240
GAAAACTCTG GGCATTTCTG TTGAGCACCT TGTGGAGGGG CTAAGGAAGT GTGTAAATGA 300
GCTGGGACCA GAGGCTTCTG AAGCTGTGAA GAAACTGCTG GAGGCGCTAT CACACTTGGT 360
GTGACATCAA GATAAAGAGC GGAGGTGGAT GGGGATGGAA GATGATGCTC CTATCCTCCC 420
TGCCTGAAAC CTGTTCTACC AATTATAGAT CAAATGCCCT AAAATGTAGT GACCCGTGAA 480
AAGGACAAAT AAAGCAATGA ATACATT 507

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 519 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SE ID:5:

GAATTCGAAT TCGAATTCGT GGAACCACTG GC GA TTTTGCTAGA TTTTTCTGAT 60
TTTTAAACTC CTGAAAAATA TCCCAGATAA CT AA GCTGGTAACT ATCTTCCTGC 120
TGGTGACCAT CAGCCTTTGT AGTTACTCTG CT TT CCTCATCAAC AAAGTGCCCC 180
TTCCTGTTGA CAAGTTGGCA CCTTTACCTC TG AT TCTTCCCTTT ATGGATCCAT 240
TAAAGCTTCT TCTGAAAACT CTGGGCATTT CT GCA CCTTGTGGAG GGGCTAAGGA 300
AGTGTGTAAA TGAGCTGGGA CCAGAGGCTT CT TGT GAAGAAACTG CTGGAGGCGC 360
TATCACACTT GGTGTGACAT CAAGATAAAG AG TG GATGGGGATG GAAGATGATG 420
CTCCTATCCT CCCTGCCTGA AACCTGTTCT AG A GATCAAATGC CCTAAAATGT 480
AGTGACCCGT GAAAAGGACA AATAAAGCAA TG T 519

(2) INFORMATION FOR SEQ ID :

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGCTCGGAAT TCCGAGCTTG GATCCTCTAG AGC CGCC GACTAGTGAG CTCGTCGACC 60
CGGGAATT 68

(2) INFORMATION FOR SEQ ID NO 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AATTAATTCC CGGGTCGACG AGCTCACTAG TCGGCGGCCG CTCTAGAGGA TCCAAGCTCG 60
GAATTCCG 68

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCGGATAAC AATTTCACAC AGGA 24

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGTAAAACGA CGGCCAGT 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGTGGA[illegible] CTAAGGAAGT 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCACGGGTCA CTACATTTTA 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGCTACTGC CTTCCTCATC 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATCTTCCATC CCCATCCAC 19

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Lys Leu Val Thr Ile Phe Leu Leu Val Thr Ile Ser Leu Cys Ser
 1               5                  10                  15
Tyr Ser Ala Thr Ala Phe Leu Ile Asn Lys Val Pro Leu Pro Val Asp
            20                  25                  30
Lys Leu Ala Pro Leu Pro Leu Asp Asn Ile Leu Pro Phe Met Asp Pro
        35                  40                  45
Leu Lys Leu Leu Leu Lys Thr Leu Gly Ile Ser Val Glu His Leu Val
    50                  55                  60
Glu Gly Leu Arg Lys Cys Val Asn Glu Leu Gly Pro Glu Ala Ser Glu
65                  70                  75                  80
Ala Val Lys Lys Leu Leu Glu Ala Leu Ser His Leu Val
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Asn Lys Val Pro Leu Pro Val Asp Lys Leu Ala Pro Leu Pro Leu Asp
 1               5                  10                  15
Cys
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Asp Asn Ile Leu Pro Phe Met Asp Pro Leu Lys Leu Cys
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Leu Leu Lys Thr Leu Gly Ile Ser Val Glu His Leu Val Glu Gly Leu
 1               5                  10                  15
Cys
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:

Leu Arg Lys Cys Val Asn Glu Leu Gly Pro Ala Ser Glu Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Glu Ala Ser Glu Ala Val Lys Lys Leu Leu Gly Ala Leu Ser His Leu
1 5 10 15
Val Cys

We Claim:

1. A method of detecting the presence of a target LU103 polynucleotide in a test sample, comprising:

(a) contacting said test sample with at least one LU103-specific polynucleotide or complement thereof; and

(b) detecting the presence of said target LU103 polynucleotide in the test sample, wherein said LU103-specific polynucleotide has at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

2. The method of claim 1, wherein said target LU103 polynucleotide is attached to a solid phase prior to performing step (a).

3. A method for detecting mRNA of LU103 in a test sample, comprising:

(a) performing reverse transcription with at least one primer in order to produce cDNA;

(b) amplifying the cDNA obtained from step (a) using LU103 oligonucleotides as sense and antisense primers to obtain LU103 amplicon; and

(c) detecting the presence of said LU103 amplicon in the test sample, wherein the LU103 oligonucleotides utilized in steps (a) and (b) have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

4. The method of claim 3, wherein said test sample is reacted with a solid phase prior to performing one of steps (a), (b), or (c).

5. The method of claim 3, wherein said detection step comprises utilizing a detectable label capable of generating a measurable signal.

6. A method of detecting a target LU103 polynucleotide in a test sample suspected of containing said target, comprising:

(a) contacting said test sample with at least one LU103 oligonucleotide as a sense primer and with at least one LU103 oligonucleotide as an anti-sense primer and amplifying to obtain a first stage reaction product;

(b) contacting said first stage reaction product with at least one other LU103 oligonucleotide to obtain a second stage reaction product, with the proviso that the other LU103 oligonucleotide is located 3' to the LU103 oligonucleotides utilized in step (a) and is complementary to said first stage reaction product; and

(c) detecting said second stage reaction product as an indication of the presence of the target LU103 polynucleotide, wherein the LU103 oligonucleotides utilized in steps (a) and (b) have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

7. The method of claim 6, wherein said test sample is reacted with a solid phase prior to performing one of steps (a), (b), or (c).

8. The method of claim 6, wherein said detection step comprises utilizing a detectable label capable of generating a measurable signal.

9. The method of claim 8, wherein said detectable label is reacted to a solid phase.

10. A test kit useful for detecting LU103 polynucleotide in a test sample, comprising a container containing at least one LU103 polynucleotide having at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

11. A purified polynucleotide or fragment thereof derived from an LU103 gene, wherein said polynucleotide is capable of selectively hybridizing to the nucleic acid of said LU103 gene and has at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

12. The purified polynucleotide of claim 11, wherein said polynucleotide is produced by recombinant techniques.

13. The purified polynucleotide of claim 11, wherein said polynucleotide is produced by synthetic techniques.

14. The purified polynucleotide of claim 11, wherein said polynucleotide comprises a sequence encoding at least one LU103 epitope.

15. A recombinant expression system comprising a nucleic acid sequence that includes an open reading frame derived from LU103 operably linked to a control sequence compatible with a desired host, wherein said nucleic acid sequence has at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

16. A cell transfected with the recombinant expression system of claim 15.

17. A cell transfected with a nucleic acid sequence encoding at least one LU103 epitope, wherein said nucleic acid sequence is selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

18. A composition of matter comprising an LU103 polynucleotide or fragment thereof, wherein said polynucleotide has at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, and fragments or complements thereof.

19. The test kit of claim 10 further comprising a container with tools useful for collection of said sample, wherein the tools are selected from the group consisting of lancets, absorbent paper, cloth, swabs and cups.

20. A gene or fragment thereof which codes for an LU103 protein which comprises an amino acid sequence with at least 50% identity with SEQUENCE ID NO 14.

21. A gene or fragment thereof comprising DNA having at least 50% identity with SEQUENCE ID NO 4 or SEQUENCE ID NO 5.

```
>1235095  GAATTCGTGG AACCACTGGC TTGGTGGATT TTGCTAGATT TTTCTGATTT
Consensus GAATTCGTGG AACCACTGGC TTGGTGGATT TTGCTAGATT TTTCTGATTT

>1235095  TTAAACTCCT GAAAAATATC CCAGATAACT GTCATGAAGC TGGTAACTAT
Consensus TTAAACTCCT GAAAAATATC CCAGATAACT GTCATGAAGC TGGTAACTAT

>1235095  CTTCCTGCTG GTGACCATCA GCCTTTGTAG TTACTCTGCT ACTGCCTTCC
>1235531  TGCT ACTGCCTTCC
Consensus CTTCCTGCTG GTGACCATCA GCCTTTGTAG TTACTCTGCT ACTGCCTTCC

>1235095  TCATCAACAA AGTGCCCCTT CCTGTTGACA AGTTGGCACC TTTACCTCTG
>1235531  TCATCAACAA AGTGCCCCTT CCTGTTGACA AGTTGGCACC TTTACCTCTG
Consensus TCATCAACAA AGTGCCCCTT CCTGTTGACA AGTTGGCACC TTTACCTCTG

>1235095  GACAACATTC TTCCCTTTAT GGATCCATTA AAGCTTCTTC TGAAAACTCT
>1235531  GACAACATTC TTCCCTTTAT GGATCCATTA AAGCTTCTTC TGAAAACTCT
Consensus GACAACATTC TTCCCTTTAT GGATCCATTA AAGCTTCTTC TGAAAACTCT

>1235095  GGGCATTTCT GTTGAGCAC
>1235531  GGGCATTTCT GTTGAGCACC TTGTGGAGGG GCTAAGGAAG TGTGTAAATG
>1379417                                    AAGGAAG TGTGTAAATG
Consensus GGGCATTTCT GTTGAGCACC TTGTGGAGGG GCTAAGGAAG TGTGTAAATG

>1235531  AGCTGGGACC AGAGGCTTCT GAAGCTGTGA AGAAACTGCT GGAGGCGCTA
>1379417  AGCTGGGACC AGAGGCTTCT GAAGCTGTGA AGAAACTGCT GGAGGCGCTA
Consensus AGCTGGGACC AGAGGCTTCT GAAGCTGTGA AGAAACTGCT GGAGGCGCTA

>1235531  TCACACTTGG TGTGACATCA AGATAAAGAG CGGAGGTGGA TGGGGATGG
>1379417  TCACACTTGG TGTGACATCA AGATAAAGAG CGGAGGTGGA TGGGGATGGA
Consensus TCACACTTGG TGTGACATCA AGATAAAGAG CGGAGGTGGA TGGGGATGGA

>1379417  AGATGATGCT CCTATCCTCC CTGCCTGAAA CCTGTTCTAC CAATTATAGA
Consensus AGATGATGCT CCTATCCTCC CTGCCTGAAA CCTGTTCTAC CAATTATAGA

>1379417  TCAAATGCCC TAAAATGTAG TGACCCGTGA AAAGGACAAA TAAAGCAATG
Consensus TCAAATGCCC TAAAATGTAG TGACCCGTGA AAAGGACAAA TAAAGCAATG

>1379417  AATACATT
Consensus AATACATT
```

FIG.1

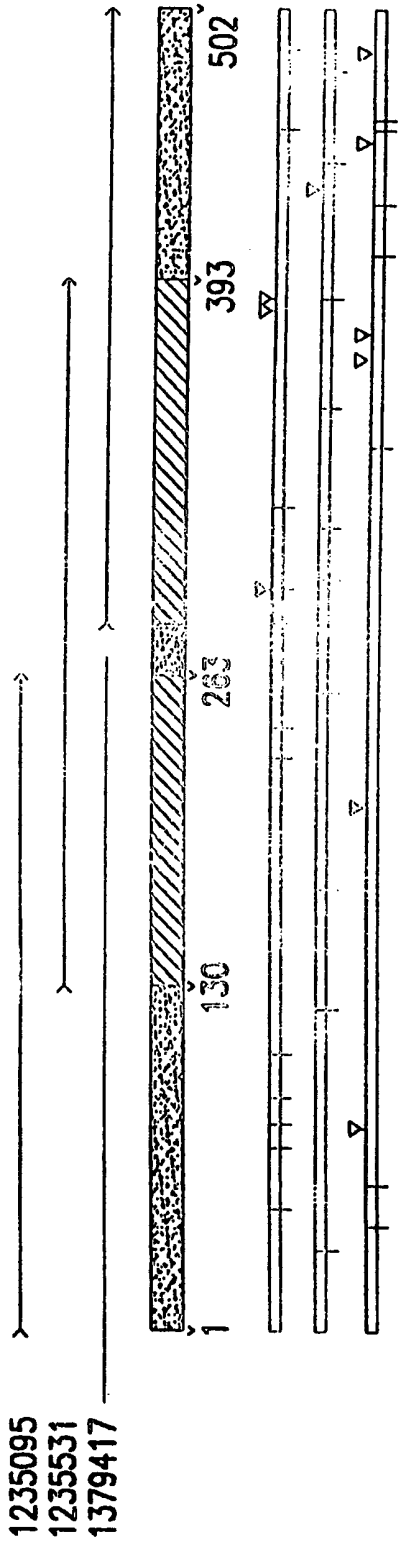
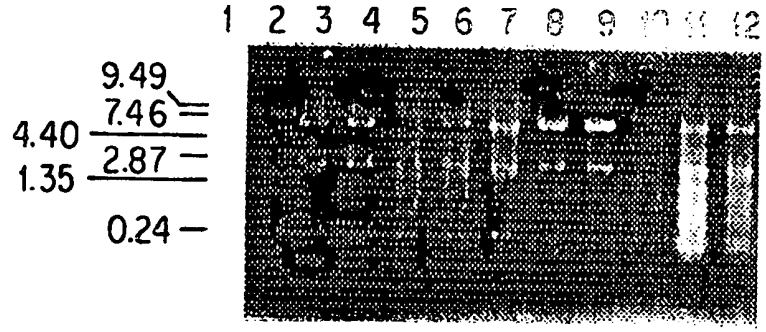
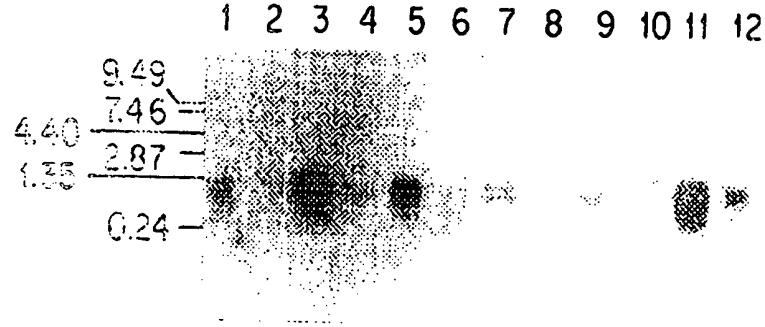


FIG. 2

Lane	Tissue
1	Bladder
2	Brain
3	Breast
4	Colon
5	Kidney
6	Lung
7	Muscle
8	Ovary
9	Placenta
10	Prostate
11	Spleen
12	Testis

FIG.3A





Lane	Tissue
1	Normal Lung
2	Normal Lung
3	Normal Lung
4	Normal Lung
5	Normal Lung
6	Normal Lung
7	Cancer Lung
8	Cancer Lung
9	Cancer Lung
10	Cancer Lung
11	Cancer Lung
12	Cancer Lung

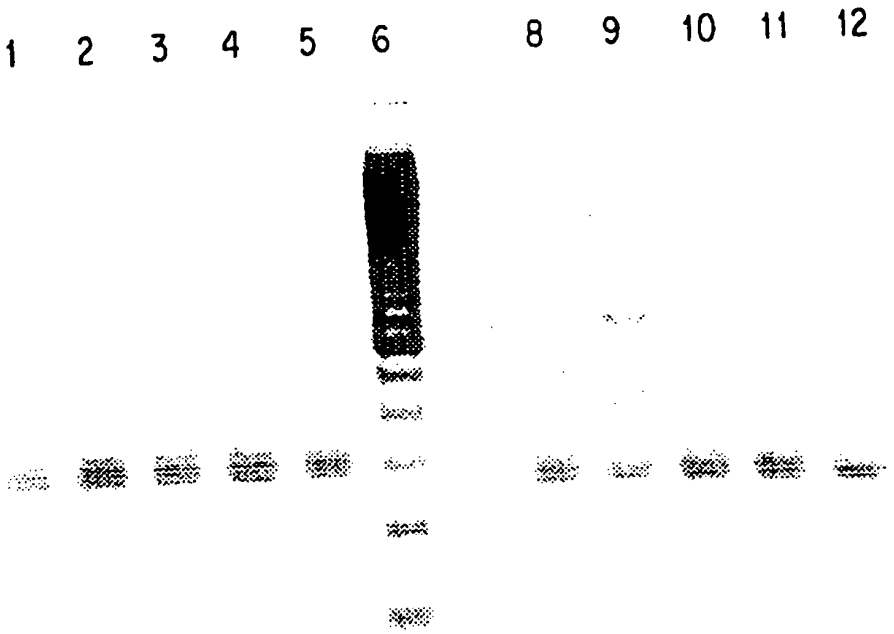


FIG. 4

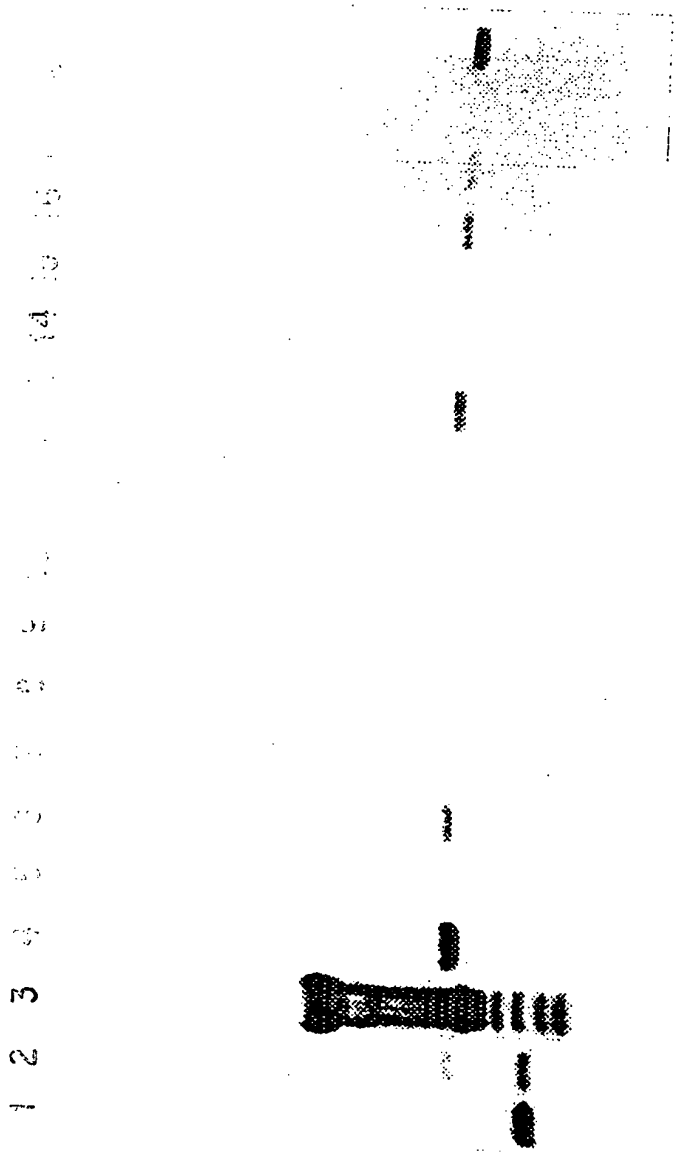


FIG. 4B

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 97/20680

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/63 C12N5/10 C12Q1/68 C07K14/47 //C07K16/30, G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL AC:W40141; ID: HS141342 Fetal lung NbH119W Homo sapiens cDNA clone, 21 May 1996 HILLIER ET AL.: "The WashU-Merck EST Project" XP002059613 see abstract ---	1-21
X	DATABASE EMBL AC:W17168; ID:HS168332 Fetal lung NbH119W Homo sapiens cDNA clone, 4 May 1996 HILLIER ET AL.: "The WashU-Merck EST Project" XP002059614 see abstract --- -/--	1-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 1998	01/04/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

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INTERNATIONAL SEARCH REPORT

Intern[illegible]nal Application No
CT/US 97/20680

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passage	Relevant to claim No
X	DATABASE EMBL AC:D30961; ID:HSL11877, 9 February 1995 SUDO ET AL.: "2058 expressed ESTs from a human fetal lung cDNA library" XP002059615 see abstract	1-21
A	WO 95 20681 A (INCYTE PHARMA INC) 3 Augu[illegible] 1995 see the whole document	1-21
A	WO 96 30389 A (MILLENIUM PHARM INC) 3 October 1996 see the whole document	1-21
A	SCHWARTZ M K: "CURRENT STATUS OF TUMOUR MARKERS" SCANDINAVIAN JOURNAL OF CLINICAL & LABORATORY INVESTIGATION, vol. 55, no. SUPPL. 221, 1 January 1995, pages 5-14, XP000490502 see the whole document	1-21

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/20680

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9520681	A	03-08-95	AU	1694695 A	15-08-95
			BG	100751 A	31-07-97
			CA	2182217 A	03-08-95
			CN	1145098 A	12-03-97
			CZ	9602189 A	14-05-97
			EP	0748390 A	18-12-96
			FI	962987 A	26-09-96
			JP	9503921 T	22-04-97
			LV	11696 B	20-08-97
			NO	963151 A	27-09-96
			PL	315687 A	25-11-96
			HU	75550 A	28-05-97
WO 9630389	A	03-10-96	US	5633161 A	27-05-97
			AU	5437896 A	16-10-96
			CA	2216717 A	03-10-96
			EP	0817792 A	14-01-98
			US	5674739 A	07-10-97